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THE LIFE WORK OF SIR JOHN SIMON.

THE death on July 23rd in the 88th year of his age of John Simon marks the passing of a great man in the history of Disease Prevention. He was a cultured man, of great strength of character, and a writer of terse and eloquent English. These qualities enabled him to influence public opinion to an extent which justifies the statement, that he did more than any other writer and worker of the Victorian era to stay the ravages of disease and to ameliorate the conditions of our national life.

To the end he retained an active interest in his life work, and the first number of this *Journal* contained a letter from him dated Oct. 22, 1900, in which while congratulating the editors on the work "you are about to commence, and on the moment you have chosen for commencing it," he regrets his inability to be a contributor to its first number, and refers to the "serious disqualification that during the last few months increasing blindness has rendered me incapable of guiding my pen for more than the signature of my name." In the same letter he alludes to the fact that "the past two centuries, and especially the last fifty years, have been beyond measure progressive in the departments of knowledge to which your undertaking relates." Now that the writer has passed away, posterity will associate the name of Simon with the most important part of this progress.

Simon began the study of medicine in 1833, when he was a few days short of 17. Two years after he obtained his M.R.C.S. he was appointed Assistant-Surgeon at King's College Hospital (in 1840). In 1845 he presented a communication to the Royal Society on the comparative anatomy of the thyroid gland, which won him the F.R.S. In 1847 he was appointed Lecturer on Pathology at St Thomas's Hospital, and subsequently became a surgeon on its staff. He stipulated for the retention of this post when in 1855 he was appointed the first medical officer to the Privy Council.

Simon clung to his surgical work, although he was not engaged in private practice; and there can be no doubt that by this means he secured a wider standpoint and a more satisfactory perspective for his public health work than if he had been restricted to the latter.

In 1848, when Simon was only 32 years old, came the turning-point in his career. The Corporation of the City of London applied to Parliament for special local powers, including the power to appoint a medical officer of health, and Simon was appointed to the post. The circumstances of the time gave prominence to the position. No other part of the metropolis had a medical officer, and only one provincial town, Liverpool. On this as on other occasions the fear of cholera was a powerful motive agent of sanitary reform. Simon held this appointment for seven years. The duties were almost undefined. He had to create them for himself. The results were embodied in reports which can be read to-day with the greatest interest. The demand for them was so great that in 1854 they were reprinted, a touching dedication to his father being prefixed, in which he states that the dedication is made "looking less to what intrinsic merit the reports may have, than to the years of anxious labour they represent: deeming it fit to associate my father's name with a record of endeavours to do my duty."

In the preface to the reprinted reports he lays "no claim to the merit of scientific discovery"; but considers it to have been "no unworthy object, that confining myself often to almost indisputable topics—to truths bordering on truism,—I should labour to make trite knowledge bear fruit in common application." After an eloquent denunciation of social evils among the poor, he adds: "I wish emphatically to declare my conviction, that such evils as I denounce are not the more to be tolerated for their rising in unwilling Pauperism, rather than in willing Filth; yet I doubt whether poverty be so important an element in the case as some people imagine.........I have no hesitation in saying that sanitary mismanagement spreads very appreciable evils high in the middle ranks of society; and from some of the consequences, so far as I am aware, no station can call itself exempt." He adds: "The fact is, that except against wilful violence, life is very little cared for by the law. Fragments of legislation there are indeed in all directions: enough to establish precedents—enough to testify some half-conscious possession of a principle: but, for usefulness, little beyond this." Simon, during the next twenty years, was destined to be a chief means of remedying this state of matters. And in this same preface he indicates the scientific requirements for improvement. "If, as is rumoured, the

approaching reconstitution of the General Board of Health is (after the pattern of the Poor Law Board) to give it a Parliamentary President, that member of the Government ought to be open to challenge in respect of every matter relating to health. What, for this purpose, might be the best subordinate arrangement of such a Board it would take a volume to discuss. But at least as regards its constituted head, sitting in Parliament, his department should be, in the widest sense, *to care for the physical necessities of human life.* Whether skilled coadjutors be appointed for him or not; engineers—lawyers—chemists—pathologists; whether he be, as it were, the foreman of this special jury, or according to the more usual precedent of our public affairs, collect advice on his own responsibility, and speak without quotation of other authority than himself, his voice, unless the thing is to be a sham, must represent all these knowledges."

The whole preface to these reports to the City of London needs to be read to be appreciated. He ends it by expressing his opinion that "there is no attachment to the incongruities I have sketched as belonging to our abortion of a sanitary system, still less is there any want of feeling for the poor......Knowledge and method and comprehensiveness are wanted—the precise, definite, categorical impulses of a Parliamentary leader, who can recognise principles and stick to them. And for such a minister, what a career!"

The writer of such masterly terse English, who had done so much for the City of London, was clearly indicated for the post of first medical officer to the General Board of Health, and in 1854 he became the first medical adviser of the Central Government.

Simon's subsequent work is embodied and to some extent buried in the annual reports of the central department to which he was attached, and would have remained so but for the enterprise of the Sanitary Institute, which in 1887 published a reprint of the more important portions of the reports, taken from blue books, etc., long out of print. For this task they fortunately secured the services of Dr E. C. Seaton, and in the two handsome volumes issued by the Sanitary Institute the most characteristic writings of Simon can be studied.

The most voluminous of these reports deals with the history and practice of vaccination. The following characteristic remark from the last page of this report is as apposite now as when written in 1857:

"No truth can be thought of, against which someone does not rail. And it would be idle to hope, under existing conditions of the human mind, that vaccination should be much more generally credited than it

is. Perhaps in no age of the world, proportionately to its institution, have persons been readier than now to accept physical marvels, and to modify their conception of physical laws, at the cajoling of quacks and conjurers. It goes with this credulity to be incredulous. Alike in rejecting what is known, and in believing what is preposterous, the rights of private foolishness assert themselves. It is but the same impotence of judgment, which shrinks from embracing what is real, and lavishes itself upon clouds of fiction."

In his "Papers relating to the Sanitary State of the People of England" Simon, commenting on Dr Greenhow's special reports, deals with the main causes of death other than old age. He illustrates the conclusion that "local excesses of fatality are due to local circumstances of aggravation; that these aggravating local circumstances are such as it is fully possible to counteract; and that of the total mortality ascribed to these influences in England a very large share is preventable." Again, "in the districts which suffer high diarrhoeal death-rates, the population either breathes or drinks a large amount of putrefying animal refuse." Fever is "essentially a disease of filth."

So far as tubercular disease is concerned, "in proportion as the male and female populations are severally attracted to indoor branches of industry, in such proportion, other things being equal, their respective death-rates by phthisis are increased." Again: "it cannot be too distinctly recognised that a high local mortality of children must almost necessarily denote a high local prevalence of those causes which determine a degeneration of race." He sums up by expressing his conviction that "the vast range of that aggregate mortality in different districts of England is due to the varying prevalence of two local causes:—First, to differences of degree in common sanitary defects...... and secondly to occupational differences among the inhabitants," especially as bearing on the feeding of children. Simon concludes from the preceding review of the sanitary condition of the people that first of all the people must become "fully informed," and secondly "publicly informed" of the existence and curability of these evils. The precise facts must be laid before "the local public and the general public and the government and the legislature"; and that every local authority "must be properly advised by skilled officers as to the special causes of disease operating within their respective jurisdictions." This report issued in 1858 peavd the way for reforms of the greatest importance.

Alongside direct public health work, Simon brought to his aid the best scientific aid in the investigation of the causation of disease. Burdon Sanderson's important work on the Pathology of the Infective

Processes, on Infective Inflammations, and on the Infective Character of Tuberculosis consisted of investigations initiated in the Medical Department of the Privy Council or Local Government Board while Simon was Medical Officer. The following extract from one of Dr Sanderson's reports shows how nearly he anticipated Koch's eventual demonstration:

"As regards the question of a specific contagium of tubercle, we think it very important to note that this is not as yet disproved by the facts of traumatic tuberculosis. It still remains open to inquiry whether or not injuries which are of such a nature that air is completely excluded from contact with the injured part are capable of originating a tuberculous process. The results of the following experiments undertaken at the instance of Mr Simon, with special reference to this question, seem indeed to suggest that they may not be so. Setons steeped in carbolic acid were inserted in ten guinea-pigs on the 24th of Sept. 1868, each animal receiving two. At the same time extensive fractures of both scapulae were produced on five others, care being taken not to injure the integuments. No tuberculosis or other disease of internal organs resulted in either case: these facts certainly point to the necessity of further investigation in this direction." Burdon Sanderson's important conclusion that "every kind of contagium consists of particles," led Simon to the statement that such knowledge when further pursued "must sooner or later......be of the largest conceivable advantage to mankind."

In 1876, when Simon, for reasons referred to below, thought it his duty to resign his office, the specific infectivity of tuberculosis, and the question whether this infectivity was dependent on a specific organism, were matters which occupied the attention of pathologists in all parts of the world; but neither question had been settled experimentally. That this was eventually accomplished on the Continent rather than in England may perhaps be attributed to the interruption, occasioned by Simon's retirement, of the pathological research which he had initiated for the Medical Department of the Local Government Board.

It is impracticable to refer to all the important investigations carried on by the able band of helpers whom Simon gathered around him. In administrative concerns no less than in the organization of investigation he showed insight and foresight. He was the means of initiating the decennial supplements of the Registrar-General, and the occupational statistics embodied in them. His opposition to quarantine caused its gradual abolition. He organised vaccination, but when in 1876 Simon found that under the Local Government Board, created in 1871, public health was subordinated to poor-law administration, and that medical

investigation was being curtailed and hampered in all kinds of ways, he resigned, and the subsequent years of life were spent in retirement.

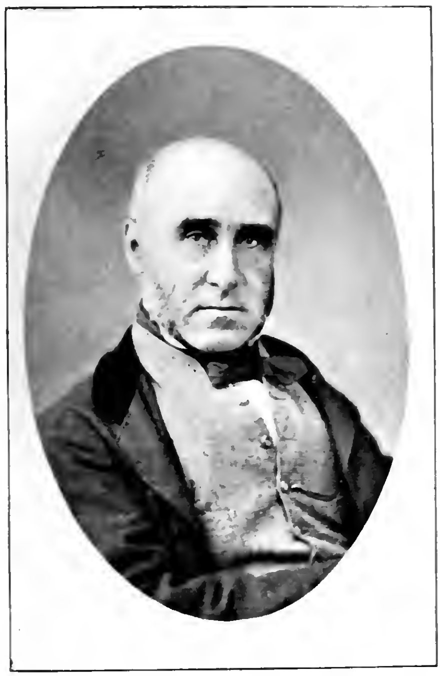
Subsequent to his retirement he wrote his work on *English Sanitary Institutions*, which is a classic of the literature of public health. The evolution of sanitary administration is discussed in this work with a wealth of knowledge and wisdom, and with a sympathy which make it an invaluable mine of information for all well-wishers of humanity. In the chapter on the "Growth of Humanity in British Politics" he traces the influence of Howard's work, of the revivalism of the Wesleys, and allied forces in causing reformation of the criminal code, the abolition of slavery, and the numerous Factory and Workshops Acts and Public Health Acts of the 19th century.

Enough has been said to justify the statement that Simon's name will ever be bound up with the history of sanitary administration in England; and that to his brilliant advocacy of reform we are indebted more than to any other single cause for our present relatively advanced position in sanitary administration.

The following list of Sir John Simon's appointments, contributions, etc., is taken from the Medical Directory:

K.C.B., M.R.C.S. Eng. 1838, F. (Hon.) 1844, Vice-Pres. 1876–8, Pres. 1878–9; F.R.S.; Hon. M.D. et Chir. Munich; Hon. M.D. Dub.; Hon. D.C.L. Oxf.; Hon. LL.D. Camb. and Edin.; late Crown Mem. Gen. Med. Counc.; Past Pres. Path. Soc. and Med. Teach. Assoc.; Cons. Surg. (formerly Surg. and Lect. on Path.) St Thos. Hosp.; formerly Off. of Health City of Lond.; Med. Off. Gen Bd. Health, Privy Counc. and Local Gov. Bd. Author of *Reports on the Sanitary State and Requirements of the City of London*, 1848–55, and on those of the *People of England*, 1855–77; *Observations on Medical Education*, 1842; "Comparative Anatomy of the Thyroid Gland," *Philos. Trans.* 1844; *Physiological Essay on the Thymus Gland*, 1845; "Subacute Inflammation of the Kidney," *Med. Chir. Trans.* 1847; *The Aims and Philosophic Method of Pathological Research*, 1847; *General Pathology*, 1850; *Introduction to Reprint of City Sanitary Reports*, 1854; *English Sanitary Institutions*, 2nd edit. 1897; Editor of *Spiritual Philosophy* of Joseph H. Green, 1865. Contrib. of Arts. "Inflammation," *Holmes's Syst. of Surg.* 1860–70; "Contagion," *Quain's Dict. of Med.* 1878–94; "On some Points of Science and Practice concerning Cancer," *Brit. Med. Journ.* 1878; "Charitable Bequests Forbidden by Law," *Ibid.* 1890; "In Memoriam," *Life of Lord Sherbrooke*, 1893; "The Ethical Relations of Early Man," *XIXth Century*, 1894.

A. N.



SIR JOHN SIMON

Born at Blackheath in 1816
Died in London, July 23, 1904

EQUINE PIROPLASMOSIS, OR "BILIARY FEVER."

By THOMAS BOWHILL, F.R.C.V.S.,
Government Veterinary Surgeon, Department of Agriculture, Cape Colony.

THE disease known as equine piroplasmosis is one possessing great importance to the farming community of certain portions of Cape Colony, especially to importers, for the reason that recently imported horses sometimes develop a very acute and often fatal form of this malady. A similar disease attacks mules. The recently reported outbreak of piroplasmosis amongst donkeys in the Transvaal, if not identical, is probably very closely allied to the malady affecting horses and mules. A great mortality amongst donkeys at Lydenburg was due to piroplasmosis. Equine piroplasmosis requires further investigation, it being unknown how the disease is disseminated, although most authorities consider that the parasite is transmitted by the agency of a tick.

Nomenclature, History, Geographical Distribution, etc.

Equine piroplasmosis, commonly known throughout South Africa as biliary fever, was first observed in Natal, in 1883, by Wiltshire, who named the malady anthrax fever. Subsequently Hutcheon described it in the Cape Colony as biliary fever of the horse—the name by which it is most commonly known at this date. Hutcheon also states, "individual cases occur all over the Colony, but it is most prevalent in the Cape Peninsula, and along the East Coast to about 110 miles inland. It may occur at any season of the year, but most frequently during the summer and autumn months. It is fully as common amongst stable-fed horses as amongst those which are never inside a stable." Guglielmi (1899) described the disease in Italy, as horse

malaria, and was the first to discover endoglobular parasites in the blood of the infected animals. Rickmann (1902) described endoglobular parasites in the blood of horses that succumbed to horse sickness, and concluded that horse sickness was similar or closely allied to pernicious malaria in man. Bowhill (1904) observed *Piroplasma equi* in a horse that died of naturally acquired horse sickness at Grahamstown (see Fig. 6).

Koch (1904), in the report on the recent horse sickness experiments in Rhodesia, states that one of his young animals developed a fatal attack of biliary fever after inoculation with blood from a salted horse, and that amongst the other experimental animals he had six cases of biliary fever.

Theiler (1902), described the disease as equine malaria, owing to the parasites in specimens he sent to Laveran being classified by that authority as *Piroplasma equi*.

Edington (1901), on the other hand, states, "I am strongly of the opinion that a disease in horses in South Africa commonly described as biliary fever is in the majority of instances naturally acquired malarial horse sickness. I believe however that a true biliary fever also exists," a very inclusive statement. According to Hutcheon, "biliary fever has also been described by veterinary surgeons in Natal as the biliary form of horse sickness—but although it bears a strong resemblance to horse sickness in some points, I am convinced that it is distinct in its origin from that disease and amenable to different treatment." Hutcheon's conclusions are as far as I am able to ascertain from the literature in my possession, accepted by all recent investigators, both here and abroad, with the exception of Edington (1901), who persists in dividing horse sickness into two forms, (1) virulent form with continued fever, "in which no parasites are visible," and (2) a non-virulent form with a malarial type of fever, "and in which parasites are found." Bruce (1902), considers Edington's so-called visible parasites of accidental occurrence as far as their relation to true horse sickness is concerned. He states, "(1) the hypothetical germ of horse sickness undoubtedly does pass through a porcelain filter—the experiment has been made by Dr John McFadyean, Principal of the Veterinary College, here in London, and by H. Watkins Pitchford and myself in Natal amongst others. (2) The phase of the germ which does not, according to Edington, pass through the filter is in my opinion not the germ of horse sickness at all, but a totally different well-known parasite, the *Pyrosoma equi*, which Edington evidently

accidentally met with when working at horse sickness, and thought a phase in the life history of the parasite of horse sickness." Bruce's contention is supported by the afore-mentioned association of biliary fever parasites with true horse sickness by Koch, Rickmann, and Bowhill. It is also important to note that Theiler (1902), who studied equine malaria in a horse sickness district, mentions no co-relation between equine malaria and horse sickness, but describes the former as a distinct type of disease, thus qualifying Hutcheon's statement, "I am convinced that it is distinct in its origin." It is a tribute to Hutcheon's powers of observation, that a view promulgated a long time ago from purely clinical observation is now corroborated by scientific research, and accepted by all investigators capable of determining an issue of this nature.

Finally, Ziemann (1902) has described equine piroplasmosis as occurring in Germany, and states that a similar disease prevails in Venezuela.

Cause of the disease:
The Parasite and probable agent of transmission.

The parasite causing piroplasmosis of the horse, mule, and donkey, belongs to the haemocytozoa or endoglobular haematozoa.

Of these haemocytozoa the genus Piroplasma is the most important to veterinary surgeons as parasites of this genus cause devastating diseases amongst domesticated animals in many parts of the world. The various known species and their hosts are enumerated by Nuttall (1904).

The parasite found in the donkey in the Transvaal was discovered by Theiler (p. 316), who states "it is very closely related to if not identical with the Piroplasma found in malarial or biliary fever of the horse and mule."

By what means the parasites are transmitted to horses, mules, and donkeys, is at present unknown, but judging by analogy it seems probable that the parasites are conveyed by species of ticks, as has been amply demonstrated experimentally in the cases of bovine and canine piroplasmoses. I have usually found a great number of the common blue ticks (*Rhipicephalus decoloratus*) present on the infected animals that have come under my notice. This species of tick is known to transmit the *Piroplasma bovis* in South Africa. Theiler also considers this tick as probably responsible for the propagation of equine piroplasmosis.

The Parasite.

In prepared blood films, stained with a modified Romanowsky stain, Laveran's stain, or Azur II and Eosin, the parasites can be demonstrated without much difficulty when the examination is made in the earlier stages of the disease. In some cases the parasites are difficult to detect in the peripheral circulation. With any of the above-mentioned staining reagents the cytoplasma is stained blue, and the karyosome a bright red. The following is a description of the various forms of the parasite I have observed in Albany and adjoining coast districts.

1. *Large and small spherical forms* (sometimes of varying sizes in the same corpuscle), the latter about half the diameter of the former, the karyosome being situated at the edges of the parasite in both forms. See Figs. 1, 2, and 8.

2. *Large and small pyriform parasites*, single and in pairs.

3. *Large and small rod-like bodies*, some of them extending across the whole diameter of the corpuscle, sometimes in pairs; in one corpuscle three were observed lying parallel to each other. See Fig. 3.

4. The *Rosette* form, consisting of four bodies connected in the centre by very fine threads, each body usually containing a karyosome at the distal extremity. Some of the parasites resemble a Maltese cross, others a St Andrew's cross, and where the rosette is formed of three leaves it can be compared to the Manx coat-of-arms. In some specimens only one body has been observed to contain a red stained karyosome. The rosette form is a phase in the reproduction of the parasite which eventually divides into four, although division into two sometimes also occurs. See Figs. 4 and 5.

5. *Flagellate forms.* These bodies have been observed free (extra-corpuscular). They consist of a distinct pear-shaped head, possessing a clearly defined red stained karyosome, and a long flagellum ending in a bulbous protuberance. Flagellate forms have also been observed where the body and flagellum were situated within a blood corpuscle. In one instance the flagellum could be traced passing out of the corpuscle and terminating in a bulbous protuberance a considerable distance from the infected corpuscle. In the latter case the corpuscle measured 3μ, the length of the body of the parasite 1μ, and that of the flagellum $3{\cdot}5\mu$. See Fig. 10. In other films longer flagella were observed, but their structure was so delicate and difficult to focus, that while a faint print could be obtained from the negative, repro-

duction would not yield sufficient detail to enable the figure to be of any special interest or instructive value. See Figs. 9 and 10.

6. A great number of parasites being present in one case, some blood was drawn from the same animal under aseptic precautions into a flask containing some sterile citrate of potash solution and kept at room temperature.

Two days later, when films were prepared from this blood, the parasites, which were previously nearly all intracorpuscular and of varied shape, were now observed to be nearly all spherical or ovoid in form, extracorpuscular, and arranged in irregular masses, the karyosome being stained a bright red and the cytoplasm a light blue. See Fig. 7. The karyosome stained red up to the 4th day. Theiler (1902) observed that the parasites in incubated blood disappear as soon as the corpuscles lose their haemoglobin, and that they keep their colourability longer at room temperature. Even after being kept 12 days in an ice box he found that stained specimens showed the piroplasmata as distinctly as fresh ones, both karyosome and plasma being distinctly stained by Laveran's method; the colourability of the nucleus being an indication of its being alive. He also observed that nearly all the parasites were spherical. Similar results were not obtained with blood kept for 12 days in an incubator. The proliferation of *Piroplasma bovis* observed by Lignières (1870) in South America, was not seen by Theiler in transplantations made into fresh serum. In some experiments I made, fresh serum was added at different times to infected blood, both at room temperature and in the incubator at 29° C. up to 40° C., but no definite proliferation was observed, although a slight amoeboid movement of the parasites was noted on one or two occasions. The changes of form described by Lignières in *Piroplasma bovis,* were not observed by me in *Piroplasma equi.*

Inoculation Experiments.

Theiler (1902) failed to transmit the disease by direct inoculation, not even by the transfusion of about 1 litre of blood from the jugular vein of a sick horse into the vein of a healthy one. Koch (1904) was also unsuccessful; he states, "while fortifying our immune animals I injected intentionally on several occasions blood containing the organism of biliary fever and never succeeded in inducing an attack of the disease, but one of our young animals inoculated with 20 c.c. of blood taken from an old salted horse developed a severe and fatal attack of

biliary fever after an incubative period of nine days." Edington (1904) found that the blood obtained from donkeys previously inoculated with virulent horse sickness blood (without being severely affected) when drawn about the 10th or 11th day after inoculation and injected into clean horses was capable of setting up a fever accompanied by definitely marked remissions and intermissions. In two of these animals the blood corpuscles were found to be infected with a parasite resembling the organism present in Texas fever. In a later publication Edington states he managed to regenerate a virulent form of horse sickness from the malarial type. The disappearance of the parasites in the virulent disease, and their reappearance in the non-virulent or malarial type, as stated by Edington, seems to me highly doubtful. Spreull inoculated a sheep and a goat intravenously with negative results.

Theiler injected blood from horses suffering from equine malaria into cattle with negative results. Such blood had no effect on rabbits, dogs and guinea-pigs. Injected into the peritoneal cavity of guinea-pigs it has no effect. As I have also found, according to Nicolle and Adil-Bey, blood of cattle suffering from piroplasmosis is toxic to guinea-pigs, 1 c.c. killing these animals rapidly when injected intra-peritoneally. Guinea-pigs resist an injection of normal cattle blood in quantities up to 5 c.c.

Symptoms.

The period of incubation is unknown. Koch stated that it was nine days in one of his experimental animals. The malady occurs in an acute and a chronic form, the infected animals exhibiting the following symptoms:—

Temperature. The onset is ushered in by intense fever reaching to 104·2°—107° Fahr., in one instance I recorded 108° Fahr. In chronic cases the temperature is very fluctuating. The temperature becomes subnormal shortly before death takes place.

Prostration. In acute cases the animal hangs its head, there is increased lachrymation, disinclination to move, a stumbling gait. In the later stages there is a partial loss of power in the hind limbs, the animal becomes comatose, and death follows in a few hours.

Appetite. Sometimes the animal only picks at its food, in other cases anorexia is complete or the animal may be voracious.

Icterus and Anaemia. The mucosa of the eye, sclerotic, and other visible mucosa, also portions of the skin devoid of hair, are coloured yellow. In acute cases, the conjunctiva and nictitating membrane are

studded with numerous reddish-brown spots. This lesion was well marked amongst the transport mules during the late war on the return march from Barberton. In donkey piroplasmosis the mucosa are stated to be clean and pale, and not yellow or dirty as in the equine disease. I have also observed a blanched condition of the mucosa of the eye in this district, the diagnosis of piroplasmosis being confirmed microscopically. Anaemia is well marked in most cases, it is progressive especially in the chronic form.

Pulse. This varies according to the state of the heart, sometimes it is weak and irregular, at other times its action is tumultuous and palpitations are easily discerned.

Respiration. Accelerated at first, sometimes abdominal.

Faeces. Diarrhoea is sometimes apparent at first, in other cases the bowels are constipated, the faeces being offensive and black coloured, and covered with slime.

Urine (haemoglobinuria). The urine is usually high coloured, and in some of the mild cases an intense polyuria is sometimes observed. I have seen haemoglobinuria in three cases, all of the animals recovering.

The duration of the disease is variable; death sometimes occurs in 2—5 days. Sometimes the fever lasts for nine days when it ceases, the animal becoming convalescent a few days later. The longer the disease lasts the more uncertain is the prognosis.

Mortality. In my districts the death-rate has not been excessive, out of 150 cases that have passed through my hands during the last two years I know of only three deaths, and these were due to complications or terminal infections.

Treatment. A great many cases recover without any special treatment, some appear benefited by small doses of bicarbonate of soda in the food or drinking water. Hutcheon recommends the use of belladonna and ammonium chloride. A dose of calomel and raw linseed oil, followed next day by bicarbonate of soda and ammoniated quinine (where great depression is present), will usually be found sufficient in cases running an ordinary course. Where complications arise treatment requires to be modified according to the extent and character of the complications.

Pathology.

External appearances. The carcase is very much emaciated, the mucosa and subcutaneous tissue stained yellow, the muscles a brownish-

red with a yellowish tinge in certain parts. All the tissues are anaemic, but this condition is somewhat obscured by the icterus.

The blood is thin and watery; it coagulates quickly after death, the clot being small and soft. The serum exuding from blood kept in a test-tube is brownish-yellow in colour and contains a great many red blood corpuscles, which are deposited by degrees at the bottom of the tube. *Spleen:* enormously enlarged (may weigh up to 5 kilogrammes), capsule distended, pits slightly on pressure, pulp softened and of a tarry colour. *Liver:* Yellow coloured, congested, bile capillaries dilated and full of fluid. *Kidneys:* Frequently enlarged, anaemic, and cortex infiltrated. *Bladder:* contains usually normal urine except in cases associated with haemoglobinuria, when it is dark brown coloured and small petechiae are present on the mucosa of the bladder. *Lymphatic glands:* Those of the spleen, liver and kidneys are tumefied and haemorrhagic, and in the mesentery and sub-lumbar region there is a serous infiltration. *Stomach:* Usually empty, mucosa sometimes congested. *Intestines:* Mucosa pale coloured, swollen, and in some cases the seat of haemorrhagic patches. *Serous cavities:* The pleurae are stained yellow and there is sometimes a slight effusion into the peritoneum. In donkeys this lesion is well marked, the effusion being stated to appear like an acute dropsy (Dale). *Heart:* This organ is sometimes enlarged and flabby, small ecchymoses are sometimes observed on the parietal layer of the pericardium, small endocardial punctiform haemorrhages are usually present associated with a gelatinous infiltration, which also involves the valves and origin of the aorta. The cavities are usually filled with blood. *Lungs:* Usually normal in appearance, the anterior lobes are sometimes emphysematous, occasionally, when complications have arisen, broncho-pneumonic and allied septic changes are in evidence.

Immunity.

There is some evidence that South African veldt horses are more or less immune to this disease. My experience has led me to believe that this immunity depends upon the animal being reared in an infected area. I have seen the disease in Cape bred horses in the Albany district; imported horses are however the greatest sufferers. I am not certain that one attack produces immunity, but I am inclined to believe it does.

Secondary or terminal infections.

A catarrhal condition of the bronchi, hypostatic pneumonia, etc. arising in the course of the disease render the animals prone to secondary infections. Germs or parasites bearing no direct causal relation to the primary disease find conditions favourable for their development, and give rise to lung complications, which have no connection with the original disease *per se*. Secondary infections may also take place through the digestive tract. Secondary infections may be local or general, and consequently may give rise to conditions of great complexity, this especially when the piroplasmosis attacks an animal primarily suffering from another perhaps chronic disease which may show renewed activity.

Theiler, speaking of the sequelae of equine piroplasmosis, states, "it is exceedingly rare to find that only the Piroplasma is present in a horse suffering from or dying of biliary fever. In nearly every case I found a bacterium which was present sometimes in the blood and always in the spleen." The organism is described as a cocco-bacillus, showing bi-polar staining. It is not unlikely from the description given that the complication was a Pasteurella infection. Dale, in piroplasmosis of the donkey, observed different kinds of pneumonia, principally of a septic nature, also intestinal lesions which in some cases hastened death.

As already mentioned, I have observed a case of typical acute horse sickness (naturally acquired) in which *Piroplasma equi* was present. See Fig. 6. Again, in a case of equine piroplasmosis, I observed Filariae together with the *Piroplasma equi* in the blood. The Nematode may be the *Filaria sanguinis equi* of Sonsino or larval forms of the Filaria which causes the blood-sweat disease, "Haemathydosis" (see Fig. 8). I have similarly met with a Spirochaete in the liver of an ox that died from ordinary coast red-water. (See Fig. 15.) Theiler has observed Spirochaetes in cattle, also in the blood of a sheep and horse, in the Transvaal.

The photomicrographs which accompany this paper were made by me from specimens I have prepared and stained, and represent a selection from an extensive series. A flagellate body seen in a case of Rhodesian fever of cattle was figured in an earlier paper in this *Journal* (Vol. IV. p. 218), where flagellate bodies of similar character in the case of *Piroplasma canis* are also described and figured.

EXPLANATION OF PLATES I TO III.

Piroplasma equi.

Plate I. Figs. 1, 2 and 3. Blood film preparations. Different forms of intra-corpuscular parasites. × 1200.

Figs. 4, 5. The same as above. Forms of parasites resembling St Andrew's and Maltese crosses respectively. × 1500.

Plate II. Fig. 6. Bigeminate form of parasite seen in blood of a horse suffering from horse sickness and piroplasmosis infections combined. × 1000.

Fig. 7. Free parasites seen in citrated blood kept two days at room temperature. × 1000.

Fig. 8. Blood of a horse suffering from mixed infection: Piroplasmosis and Filariasis, three Filariae being visible in the field. × 1000.

Figs. 9, 10. Flagellate forms. × 1500.

Piroplasma canis, Piroplasma bovis, Spirochaete.

Plate III. Fig. 11. Piroplasma canis in section of dog's kidney. The parasites intra-corpuscular. × 1200.

Fig. 12. P. canis. Rosette-like grouping of intracorpuscular parasites. × 1800.

Fig. 13. Piroplasma bovis in blood of cattle. × 1500.

Fig. 14. P. bovis in spleen smear. Flagellate body. × 1200.

Fig. 15. Spirochaete in liver of cow dead of red-water. × 1200.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

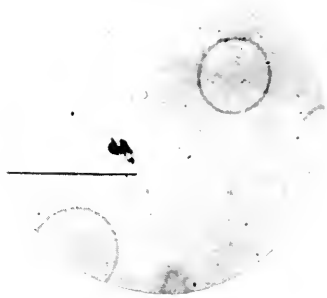

Fig. 9.

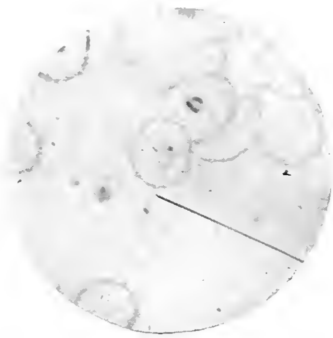

Fig. 10.

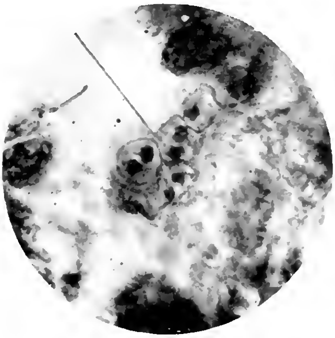

Fig. 11.

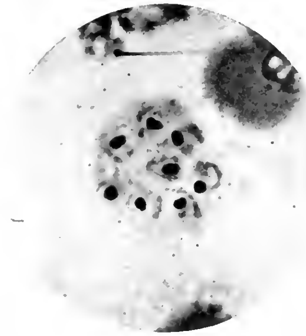

Fig. 12.

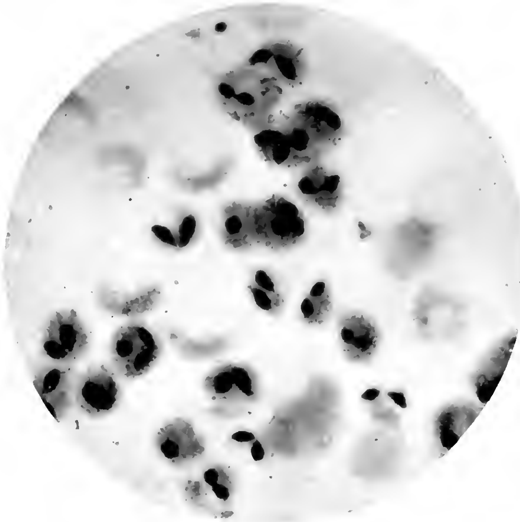

Fig. 13.

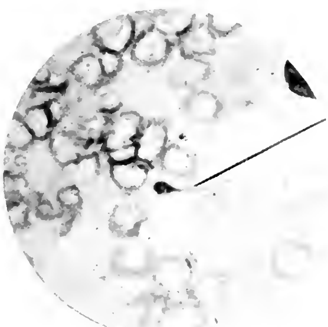

Fig. 14.

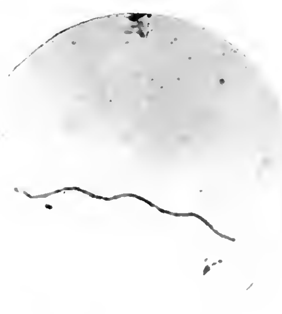

Fig. 15.

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A NOTE ON THE NATURAL OCCURRENCE OF PIROPLASMOSIS IN THE MONKEY (CERCOPITHECUS).

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During the months of May and June last three batches of monkeys (Cercopithecus), making fourteen in all, were sent to me for experimental purposes. The first lot of four monkeys arrived on May 15th, and on taking their temperatures the following evening it was found that they all had fever varying from 103·8° to 106·4° F. Blood films were taken and examined the following day, but nothing was then found save a rather marked degree of polychromatophilia. That evening, May 17th, the temperature of one of the monkeys was found to be 110·4°, the animal was moribund, and died within two hours. An autopsy was made next morning, but nothing abnormal could be found. That afternoon, in examining a fresh blood preparation, a non-pigmented pear-shaped endocorpuscular body was found. This body was single, pear-shaped when first seen, and gradually changed its shape, becoming first oval and then again pear-shaped but with the thin end now at the opposite extremity. No protrusion of pseudopodia was observed.

This discovery led to the prolonged re-examination both of the films already stained and of fresh ones. In every case, including that of the dead monkey, piroplasmic bodies were found to be present in very small numbers, usually not more than two or three being found in a large blood film. Stained by Leishman's method the commonest form seen was a round or ovoid endocorpuscular blue body with a red dot of chromatin situated nearer to the periphery than to the centre of the body. In a few cases corpuscles were found containing two of these bodies situated close together.

On June 19th six monkeys, and on June 29th four more were

received. All their temperatures were found to be high, and in every case examination of the blood showed the presence of piroplasma.

Since the discovery of the parasite very many blood examinations have been made, and it has been found that the round form of parasite is the commonest, but that distinctly pear-shaped forms also occur. The double forms are exceedingly rare, and the two parasites in this case are always almost circular. Only once again has the parasite been seen in fresh blood; the appearance and change of shape were then exactly as described above. A few extracorpuscular forms have been seen, in all respects resembling the intracorpuscular forms.

The parasites vary in size. The more common round form has a diameter usually of $1{\cdot}5\mu$. The pear-shaped forms are as a rule larger —$2{\cdot}5\mu$ by $1{\cdot}5\mu$—the largest one measured was 3μ by 2μ.

The only occasion on which the parasites were found in any number was on a day when a blood film was taken in the morning from a monkey with a normal temperature. Parasites were found to be much more numerous than usual, and it was remarked that the same evening the temperature of this monkey rose to 106·8°. But for this one occasion there has been little variation in the number of parasites found, the number remaining about the same whether the temperature be high or normal. But it must be said that this was the only time on which a blood film was obtained just before such a marked rise of temperature.

Course of the disease. Of the fourteen monkeys six have died. Two of these latter had been injected with Trypanosoma and need not be noticed; one of the other four died of hyperpyrexia, one of septicaemia following injury, and in two nothing could be found except signs of obstinate constipation. One of these two last monkeys had had faecal vomiting, and the actual cause of death was inhalation of vomit and suffocation, but in neither case could any cause for the constipation be found. Smear preparations from the organs were made in all cases except in the one showing hyperpyrexia, where the monkey died before a diagnosis had been made and before the disease was even suspected. In no case could it be said that the parasites were present in greater numbers in the smears than in the heart's blood.

The course of the disease in the three of the eight monkeys still alive is shown in the accompanying charts. Chart 1 is of a monkey of the first series, chart 2 of one of the second, and chart 3 is of one of the third series. Charts 1 and 2 show the course of the disease until the monkeys were used for other experiments, chart 3 is brought up

to date as the monkey has not so far been used for anything else. Each chart is typical of the disease in the corresponding series of monkeys. It will be seen that at first there were occasional marked rises of temperature, followed by a gradual fall extending sometimes over several days. Later on there are still sudden rises, but the fall is much more prompt. Gradually these exacerbations ceased and the temperatures became nearly normal; but slight rises to about 103° still occur and the parasite can nearly always be found in the blood. The animals' general condition has always been good, they show no loss of appetite and do not appear at all sick.

Source of the disease. All the monkeys were caught by four men in the same district (Kikuyu). After capture they were kept for a couple of days in a native village, and were then brought in to the district officer, who kept them in a cage in his compound until there were enough collected to be forwarded by train. Infection might therefore have taken place either before capture, in the native village, or at the district officer's quarters. At first I was inclined to think that the disease must have been contracted at one of the two last places, but on comparing all the charts it is seen that the temperatures of the monkeys of the third series were much lower on arrival here than were those of the second. Also, taking the charts of the third series, it is seen that they correspond closely with those of the first and second series at the same dates. It seems likely, then, that all the monkeys contracted the disease at about the same time, and this could only have been before capture.

No ticks were found on any of the monkeys nor in the crevices of the box in which the first four arrived.

The only other animals available for experimental inoculation with the blood of these monkeys were dogs. Two were injected subcutaneously with blood. The first dog, an aged pariah, showed neither rise of temperature nor parasites in the blood. The second, a mongrel terrier puppy, showed a slight rise of temperature to 103° on the nineteenth day after inoculation, but no parasites could be found in the blood, the temperature returned to normal and the dog remains in good condition.

Note. In the charts the sign + signifies a moderate number of parasites, + · parasites scanty, + · · very scanty, – signifies no parasites found.

CHART I. *Piroplasmosis in Monkeys.*

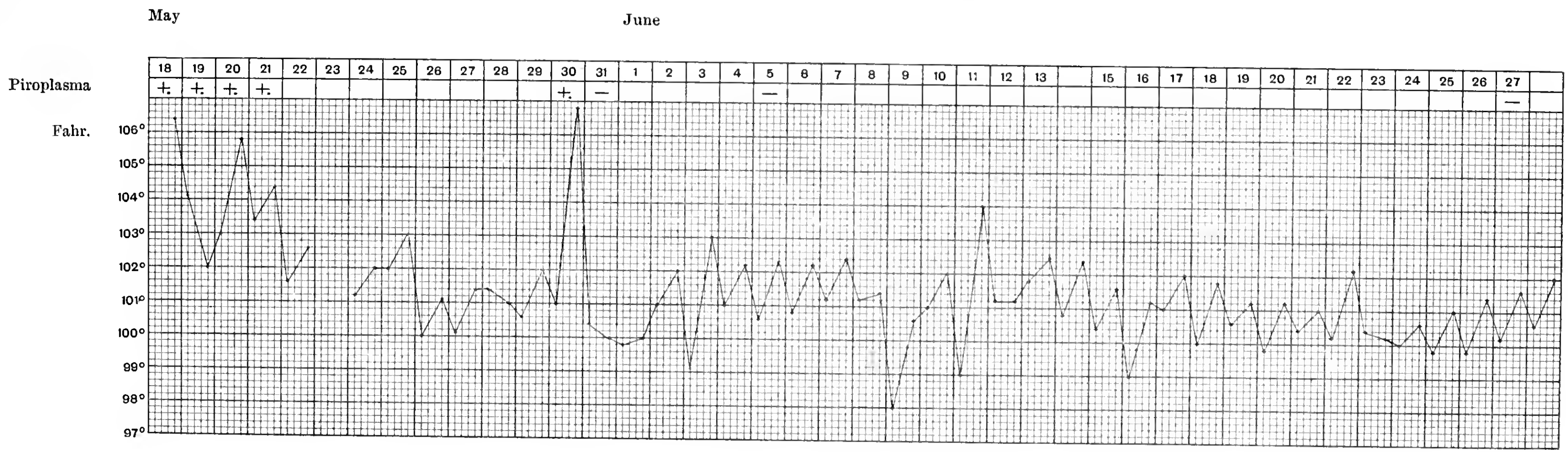
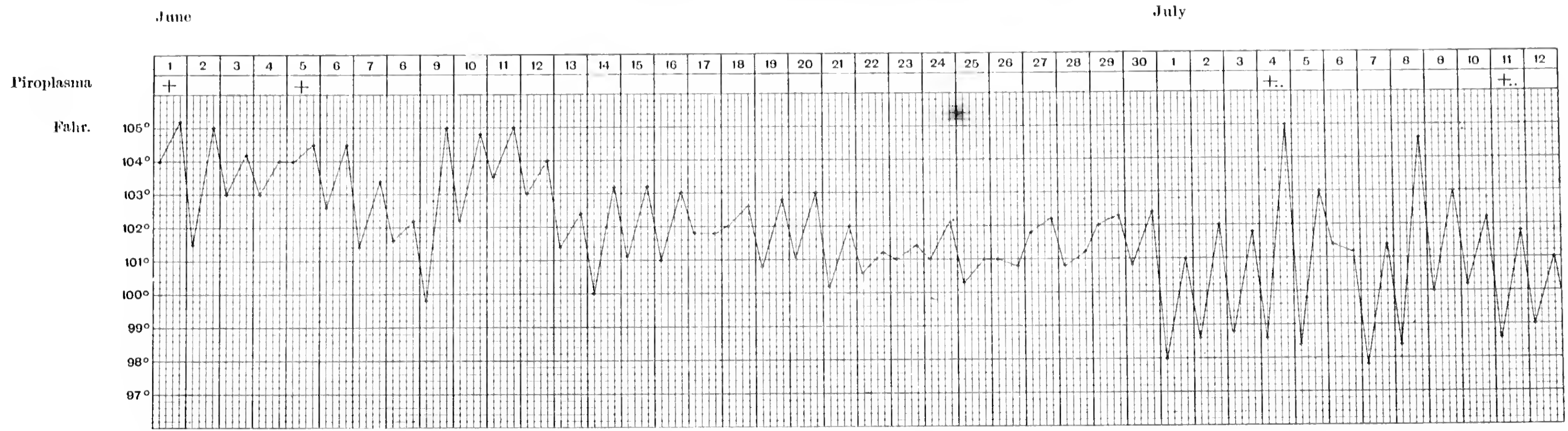
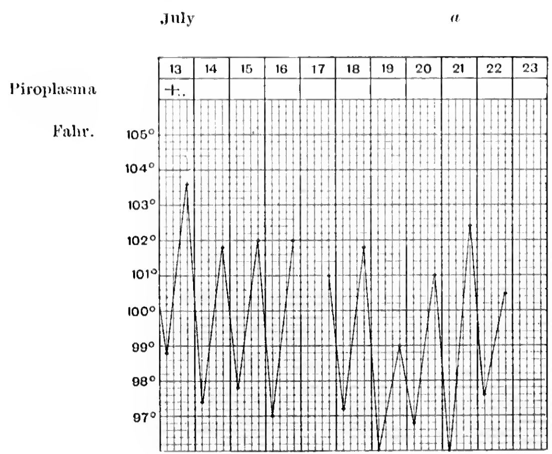


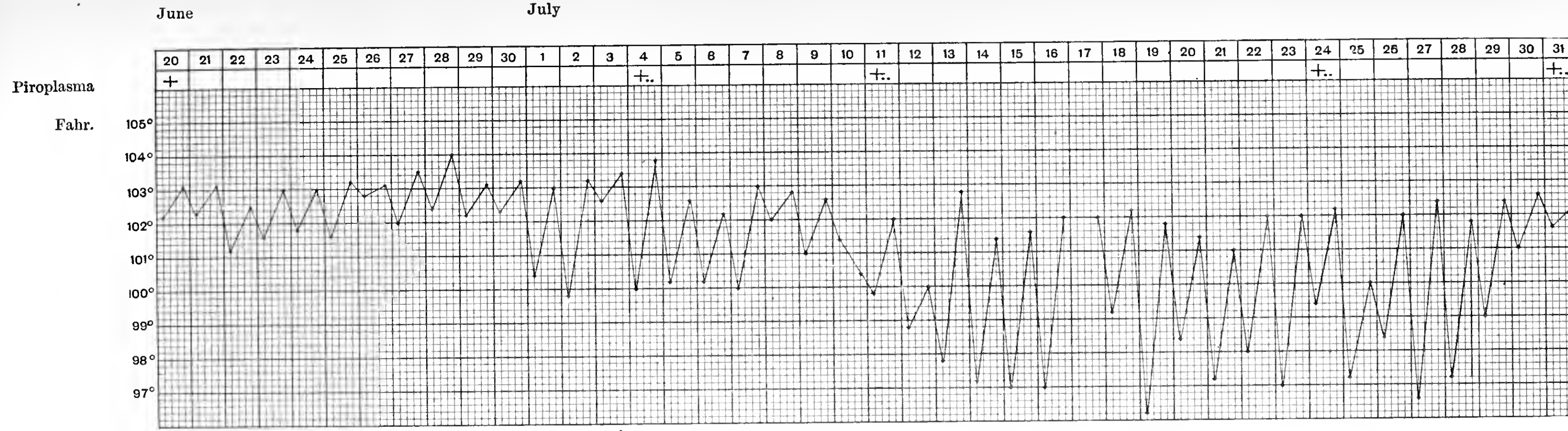
CHART II. *Piroplasmosis in Monkeys.*

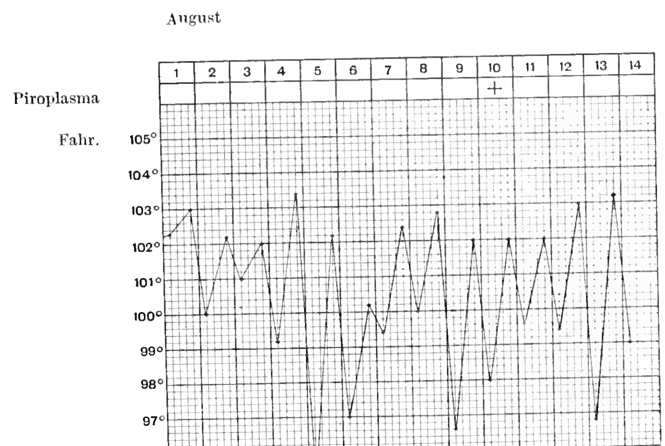




a Injected with Trypanosomata.

CHART III. *Piroplasmosis in Monkeys.*





THE CULTIVATION OF TRYPANOSOMATA.

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[Thesis for the Degree of M.D., University of Cambridge.]

(*From the Pathological Laboratory, University of Cambridge.*)

By their researches on *Trypanosoma Lewisi* McNeal and Novy (vi. '03) have shown that cultures and subcultures of this protozoan parasite can be made with almost the same ease and certainty as cultures of bacteria. Hitherto the cultivation of protozoa has been successful to a very limited degree. Kartulis (1891) claimed to have cultivated *Amoeba coli* in an infusion of straw. He found however that multiplication did not occur, unless bacteria were present. Schardinger (1896) cultivated amoebae, in the presence of bacteria, on agar to which straw or hay infusion had been added. Tsujitani (1898), using Schardinger's method, cultivated "straw amoebae" in the presence of various bacteria, e.g. *S. cholerae*, *B. typhi*. By the application of heat, the bacteria were killed; but, in the presence of the nutriment afforded by the dead micro-organisms, the amoebae continued to multiply. All attempts to cultivate the amoebae in the absence of bacteria failed. The failure of many investigators to cultivate *Tr. Lewisi*, and other members of this group of haematozoa, did not appear remarkable, in view of the difficulty of producing artificially the conditions under which these parasites exist in the blood of their hosts. A year ago Novy and McNeal announced that they had succeeded in cultivating *Tr. Brucei* and a few months later they successfully cultivated the *Surra* Trypanosome of the Philippines (see page 44). It therefore seems probable that their methods will be found applicable for the cultivation of other species of trypanosomes.

Various forms of Trypanosomiasis.

Recent discoveries have added considerably to the number of species included in the genus *Trypanosoma*. In the accompanying table (p. 26) is given a list of the chief forms of Trypanosomiasis, which are at present considered to be due to infection with distinct species of Trypanosoma. No order of the Vertebrata is exempt from this form of parasitism, and it is also probable that certain species of Trypanosomes produce morbid conditions in the blood-sucking insects, through whose agency these diseases are usually propagated. It is generally accepted that these insects act merely as "carriers" of the disease, since there is no evidence of their acting as intermediate hosts[1].

Differentiation of the various forms of Trypanosomiasis.

Until recently it was doubtful whether some of the varieties of Trypanosomiasis, mentioned in the accompanying table (p. 26), were caused by distinct species of Trypanosomes. For instance it was found to be impossible to distinguish with certainty *Nagana* from *Surra*. Minute differences in the morphology of the two parasites causing these diseases have been described, but differences almost as great exist between individual trypanosomes of the same species. The clinical symptoms are identical in the two diseases and the pathological changes, which are very few, are not distinctive. Infection experiments on animals have not proved conclusive and have caused some confusion owing to the variations in susceptibility of the same species of animal in different parts of the world. For the same reasons *Mal de Caderas* has been confused with *Dourine* and *Surra*. Immunising experiments, notably those of Nocard and of Laveran and Mesnil, have recently proved to be of great service in distinguishing these diseases. Nocard (4. v. '01, p. 466) produced a fatal infection with *Nagana* in two dogs, which had been previously immunised to *Dourine*. Laveran and Mesnil (22. vi. '03, p. 1529) have immunised a goat successively to *Nagana*, *Mal de Caderas* and *Surra*. Their experiments extended over a period of nearly two years. They reported subsequently (1904, p. 134) that this goat succumbed to an infection with the Horse Trypanosome of Gambia

[1] Schaudinn (1904, p. 387) states that *Halteridium* in the owl (*Athene noctuae*) is the sexual stage of a Trypanosome (*Tr. noctuae*, Celli and Sanfelice), which undergoes a complex form of multiplication both in the gnat (*Culex pipiens*) and in the blood of the owl, and then gives rise to the sexual forms of *Halteridium*.

Trypanosomata and their Hosts, &c.

Species of parasite	Discovered by	Host	Common name of the disease	Propagation of the disease by	Geographical distribution of the disease	Pathogenicity
Tr. of the Trout	Valentine (1841)	Salmo fario	—	—	—	—
Tr. rotatorium (Mayer)	Mayer, Gruby (1843)	Frog	—	—	World-wide	No evidence of pathogenicity for frogs or other animals.
Tr. Lewisi (Kent 1880)	Lewis (1878)	Rat	—	Fleas, ? Lice	World-wide	Only causes death of rats occasionally. A transient infection is caused in guinea-pigs.
Tr. Evansi (Steel 1885)	Evans (1880)	Horse &c.	Surra	? Tabanus tropicus, ? Stomoxys calcitrans	India, Burma, Philippine Islands, Mauritius	All domesticated animals, rats, monkeys and most other wild animals.
Tr. cobitis	Mitrophanov (1883)	Mudfish (*Cobitis fossilis*)	—	—	—	—
Tr. avium	Danilewsky (1885)	Birds	—	—	—	—
Tr. Brucei (Plimmer and Bradford 1899)	Bruce (1895)	Horse &c.	Nagana or Tsetse-fly disease	Glossina morsitans	S. Africa, W. Africa	Similar to Surra.
Tr. equiperdum (Doflein 1901)	Rouget (1896)	Horse	Dourine, Mal du coit	coitus	S. Europe, N. Africa	Similar to Surra.
Tr. —	Nepveu (1898)	Man	?	—	Algiers	—
Tr. equinum (Vosges 1901)	Elmassian (1901)	Horse	Mal de Caderas	? Tabanus, ? Stomoxys calcitrans	S. America	Similar to Surra.
Tr. gambiense (Dutton 1902)	Forde & Dutton (1901)	Man	Trypanosome fever. Now regarded as an early stage of Human Trypanosomiasis	? Glossina palpalis, ? Tabanus dorsovitta	Gambia	Monkeys, guinea-pigs, rabbits, rats, dogs, sheep, goats, etc.
Tr. ugandense (Castellani 1903) Tr. Castellani (Kruse 1903)	Castellani (1902)	Man (Uganda)	Sleeping-sickness. Now regarded as the late stage of Human Trypanosomiasis	Glossina palpalis	Uganda, Congo Free State	Similar to Tr. gambiense.
Tr. Theileri (Laveran, Bruce, 1902)	Theiler (1902)	Cattle	Gall-sickness	Hippobosca rufipes	S. Africa	Cattle only.
Tr. transvaaliense (Laveran 1903)	Theiler (1902)	Cattle	Similar to, if not identical with, Gall-sickness	—	Transvaal	Similar to Gall-sickness.
Tr. dimorphon (Dutton and Todd)	Dutton and Todd (1903)	Horse	—	? Glossina palpalis	Gambia	Similar to Surra.

(*Tr. dimorphon*). Similarly Lignières (1902, p. 112) has produced a fatal infection with *Mal de Caderas* in two dogs, which had been immunised to *Dourine*, and Nocard, Vallée and Carré (19. x. '03, p. 624) have infected a cow, which was immune to *Nagana*, with *Surra*. The cultural methods, introduced by McNeal and Novy, are likely to prove of great value in distinguishing one species of Trypanosome from another. These workers have already been able to thus confirm the results of the immunising experiments (mentioned above) which indicated that *Surra* and *Nagana* were distinct diseases (see p. 44).

Classification of the genus Trypanosoma.

Salmon and Stiles (1902, p. 29) give the following classification:—

Class:— *Mastigophora.*
Subclass:—*Flagellata.*
Order:— *Monadida*— Independently living organisms, but in some cases they form colonies. The number and arrangement of the flagella varies. There is sometimes an undulating membrane.
Family:— *Trypanosomidae*—Includes the genus *Trypanoplasma*, parasitic organisms resembling the *Trypanosoma* but possessing two flagella, one of which is situated at each end.
Genus:— *Trypanosoma.*
Species:— *Tr. rotatorium* (Mayer), *Tr. Lewisi*, etc.[1]

Morphology of Tr. Lewisi.

It is unnecessary to describe in detail the morphology of the adult form of this well known parasite, but on page 37 will be found a summary of the chief differences between the parasitic and cultural forms of *Tr. Lewisi*. It will be convenient, however, to consider at this point the modes of multiplication of these parasites, and to describe the morphology of the young forms, which closely resemble the cultural forms of *Tr. Lewisi*. The following account is taken from the description given by Laveran and Mesnil (1901, p. 684), who have extended the researches of Rabinowitsch and Kempner (1899) and of Wasielewski and Senn (1900). Two modes of multiplication of *Tr. Lewisi* are described.

[1] Laveran and Mesnil (1904, p. 41), in describing the cultural forms of *Tr. Lewisi*, remark on the resemblance of these organisms to the species included under the genera *Herpetomonas* (Kent) and *Crithidia* (Léger). [Fusiform parasites, possessing long flagella but *no* undulating membrane. Their centrosomes are placed *anteriorly* to the nucleus.] They consider therefore that the genus *Trypanosoma* should be included in the family *Cercomonadidae*, to which *Herpetomonas* belongs.

(1) *Longitudinal division.* The trypanosome increases in length, measures 35μ or more, and becomes three or four times as broad as usual. The nucleus and the centrosome enlarge, the latter elongating, and the two structures are approximated. Simple division (amitosis) of the nucleus occurs. The base of the flagellum thickens and divides at the same time as division of the centrosome occurs, which process may follow or precede division of the nucleus. By the splitting of the flagellum along part of its length a new flagellum is formed, which elongates rapidly. Subsequently the protoplasm divides. The two organisms, that are thus formed, are frequently very unequal in size. It is customary therefore to speak of mother cells and daughter cells. The latter may only measure a few microns in length.

(2) *Multiplication by segmentation.* The trypanosome becomes spherical, ovoid or irregularly shaped. The nucleus and centrosome divide and subdivide a variable number of times, new flagella are formed and the protoplasm indents and divides. In this way the rosettes, which are found in the blood during the early stages of infection, are formed. In Fig. 3, Plate IV. is shown a rosette forming by the process of segmentation. By further division a rosette, consisting of numerous young forms, which are arranged radially with their flagella directed outwards, is formed (see Fig. 4, Plate IV.). The young form differs from the adult form of *Tr. Lewisi* in the following respects: (1) Its body is fusiform in shape and much smaller. (2) The nucleus is more centrally placed. (3) The centrosome is placed close to, and not infrequently anteriorly to, the nucleus. (4) The undulating membrane is not developed or is too small to be distinguished.

I. *Cultivation of Tr. Lewisi.*

(1) *Preparation of media.* The medium, employed by McNeal and Novy, consisted of a mixture of agar and defibrinated blood. Neutral or slightly acid agar was used. The following method of preparing this medium is essentially the same as that described by McNeal and Novy. (*a*) Agar: To nutrient broth, prepared from bullock's heart without the addition of alkali and containing 1 % of peptone and salt, is added 2 % of agar. The agar is then cleared in the usual way, by the addition of white of egg, then placed in test-tubes and sterilized. (*b*) Defibrinated blood: The blood is collected from the heart of a rabbit, by means of sterilized Pasteur bulbs, and is then defibrinated in sterilized bottles, containing a few fragments of broken glass. (*c*) Rabbit-blood-agar:

To test-tubes, containing about 5 c.c. of melted agar, which has been cooled to about 45° C., is added an equal volume of defibrinated blood. After intimately mixing the blood and agar, the tube is slanted and the medium sets in a firm slope. Soon after solidification, the tubes must be placed in a nearly vertical position, so as to obtain a large amount of fluid of expression.

Precautions to be taken in preparing medium :—

(1) Strict asepsis must be ensured.

(2) Agar must be well cooled, otherwise the red blood corpuscles are broken up and a brown medium, containing haematin, is obtained.

(3) Fresh blood only should be used.

(2) *Inoculation of medium.* Two or three loopfuls of blood, taken from the heart of an infected rat, are added to the fluid of expression in the tubes of blood-agar. A larger quantity should be added, by means of a pipette, if the animal is not suffering from a severe infection. Subcultures are made by transferring one or two loopfuls of the fluid of the culture to a fresh tube of blood-agar.

(3) *Incubation of cultures.* McNeal and Novy found that *Tr. Lewisi* was best cultivated at a low temperature. Incubated at 37° C., cultures grew quickly but rapidly degenerated owing to the alteration of the blood-medium. Cultures, placed in the cool incubator (20—25°) or kept in the dark at room temperature (18—20° C.), contained numerous colonies at the end of three weeks' time and remained alive for several months. They state that evaporation should be prevented by sealing the tubes with rubber caps.

Viability of Tr. Lewisi in cultures.

It is apparently possible to cultivate *Tr. Lewisi* indefinitely; thus McNeal and Novy, in their first report, stated that they found no signs of degeneration in these organisms after cultivating them for nearly a year through eleven generations. They have announced since then (v. '04, p. 3) that this culture has been cultivated for twenty-six generations during a period of two years. The culture, which I made nine months ago, has been subcultivated through as many generations with similar results. The last generation multiplied rapidly and was quite as infective as the preceding ones.

Culture 1. *Incubated at* 20° C. The first culture was made by me on March 18, 1904. One drop of blood, taken from the heart of a white rat, was added to a tube of rabbit-blood-agar. The white

rat had been injected intra-peritoneally fourteen days previously with blood taken from an infected sewer rat[1]. Trypanosomes were found in the blood of the white rat on the fourth day after inoculation, and, at the time of its death, its blood was swarming with parasites. A drop of the culture, examined a few hours after it had been made, contained about six, actively motile, trypanosomes per field. On the fourth day the trypanosomes were not so numerous, some of them were pale and motionless, but the majority of them were very active. On the fifth day, a few small "agglomeration rosettes," composed of four to six trypanosomes, were found. The trypanosomes, which were loosely linked together at their posterior extremities, were arranged in a symmetrical manner. Their long flagella could be seen actively lashing at the periphery of the rosette. On the tenth day, small *colonies*, containing twenty or more trypanosomes, were numerous. Most of the organisms appeared to be attached to the centre of the colonies by their flagellar ends. The structure of the colonies will be described subsequently on p. 32. Free forms were numerous and exceedingly active. Pairs of trypanosomes, which were attached to one another by their posterior extremities, were common. On the twenty-first day the culture was swarming with colonies and free forms. At the end of eight weeks the culture contained numerous colonies of large size and many, actively motile, free forms; but a month later the trypanosomes were very granular in appearance and the majority of them were motionless.

Some sterile salt solution was added to this culture on two occasions to counteract evaporation, but no fresh blood was added in order to prolong the life of the culture.

Generation 2. Three loopfuls of culture 1, the latter being six weeks old, were added to a tube of rabbit-blood-agar. On the fourth day a drop of the culture contained several large masses of trypanosomes evidently formed by the partial disintegration of the colonies. Many of the trypanosomes were motionless. Active free forms were not numerous. On the fifteenth day there were numerous colonies, some being of large size. On the twenty-first day the culture was swarming with colonies and free forms. The remaining subcultures behaved similarly and need not be further described.

[1] Five out of thirteen rats (38·4 %), caught in a certain locality near Cambridge, were found to be infected with *Tr. Lewisi*.

Generation	2	made	from	generation	1 on forty-second day.
„	3	„	„	„	2 on fifteenth day.
„	4	„	„	„	3 on twenty-first day.
„	5	„	„	„	4 on twenty-third day.
„	6	„	„	„	5 on ninth day.
„	7	„	„	„	6 on thirty-first day.

Another culture of *Tr. Lewisi* was cultivated through a like number of generations, and numerous parallel subcultures were made either from this series or the one mentioned above.

Infection experiments on rats with cultures of Tr. Lewisi.

Rat 1. A white rat received intra-peritoneally three loopfuls of a culture of the third generation which was seventeen days old. Parasites were found in the rat's blood on the fifth, and were numerous on the tenth day.

Rat 2. A white rat was injected intra-peritoneally with six drops of a culture of the fourth generation which was thirty-one days old. Parasites were found in the blood of the rat on the second day. "Multiplication rosettes" and young forms were numerous on the fourth day. A few days later the blood was swarming with parasites.

Infection of rats with cultures belonging to another series.

Three loopfuls were injected intra-peritoneally in each case.

Rat A. A white rat was injected with a culture, which was thirty-three days old: culture belonged to the third generation of the series. Parasites appeared in the blood on the sixth day.

Rat B. A culture of fourth generation, which was thirteen days old, was used. Parasites appeared in the blood on the sixth day.

Morphology of the rat trypanosome in cultures.

(A) *Unstained preparations.* The parasites found in cultures of *Tr. Lewisi* vary greatly in size, shape and motility. The variability is especially marked in young cultures containing a large number of active free forms. In a hanging-drop, made from such a culture, the following forms may be recognised:

(1) *Exceedingly motile, slender forms.* The body of the trypanosome is bright and glistening and apparently homogeneous in structure, posteriorly it ends in a sharp pointed extremity, whilst anteriorly it is

prolonged into a stiff flagellum, which is sometimes twice the length of the body of the organism. No undulating membrane is visible and the movements of the organism appear to be due entirely to the rapid "vibratory" action of the flagellum. The excursion of the flagellum is extremely limited, but this is compensated for by the rapidity with which the side-to-side movements are made. Usually the trypanosome darts across the field in a straight line, but occasionally it moves in a circular direction. After describing the greater part of a circle, often more than once, it may dart off at a tangent. During its motion, a rapid rotatory movement of the whole parasite, on its long axis, may often be observed.

(2) *Shorter, spindle-shaped forms.* Trypanosomes similar to the first form.

(3) *Large pear-shaped forms.* The posterior extremity is broad and rounded. The body of the trypanosome tapers suddenly and ends in a long flagellum, which is thrown into a series of folds by the passage of wave-like contractions. This is probably an involution form, its movements are, as a rule, sluggish.

(4) *Small pear-shaped forms.* These are young, very active forms. The body of the trypanosome is small, rounded at one end, and tapers at the other end into a long flagellum.

(5) *Involution forms.* Spherical forms, pale or granular in appearance, are common in older cultures. They possess one or more active thin flagella, but do not usually exhibit more than a slight swaying movement.

(6) *Colonies.* All the forms, described above, may be found in the colonies of trypanosomes. The colonies increase rapidly in size, at first they consist of a few trypanosomes, grouped loosely together in the form of a "rosette." The trypanosomes are attached to one another at their anterior extremities by their flagella, which converge to the centre of the colony, and each individual has independent and very active movements, the body of the trypanosome being waved rapidly, from side to side or to and fro, by the action of its flagellum. The colony, if seen with its individuals all lying in the same plane, presents a beautifully symmetrical appearance. The smaller colonies move actively about, but the larger only possess a slight swaying motion. Colonies, composed of many hundreds of trypanosomes, are found in cultures which are two or three weeks old. The trypanosomes are grouped together in compact, almost spherical masses, which have the same symmetrical appearance as the smaller colonies. The older cells

in the central portion of the colony are generally spherical in shape and granular in appearance; at the periphery the cells are spindle-shaped and exhibit very active movements. In the central portion of the colony tangled masses of flagella, actively contracting, may sometimes be seen. In three or four weeks' time the colonies grow to an enormous size and contain many thousands of trypanosomes. These masses of trypanosomes are visible to the naked eye; they form small soft granules of a yellowish white colour. It is possible that these larger colonies are formed by the clumping together of several smaller colonies, but in the latter several centres of growth may often be observed. This arrangement of the colonies is shown clearly in Fig. 2, Plate V., which is a photograph of part of a large colony. At least five distinct, smaller, colonies, arranged eccentrically, can be seen. Fig. 1, Plate V. is from a photograph of a young colony, whose organisms are radiating from two centres. It shows the shape and comparative freedom of the individuals in a young colony. Fig. 1, Plate IV. is from a drawing of a colony, which has two centres of development. The angularity and the compressed appearance of the cells suggest that this colony was separated off from a much larger mass of trypanosomes. This colony and the larger one, shown in Fig. 2, Plate V., were found in the same film. (The figures referred to are made from stained preparations.)

(B) *Stained preparations*[1]. Certain details of the structure of the

[1] *The Staining of Trypanosoma in Cultures.*

At first I experienced considerable difficulty in obtaining well stained preparations. Failures were due to several causes: (1) The serous fluid of the blood-agar contains in suspension degenerated blood elements and small lumps of agar, so that even thin films thereof make opaque preparations, which are useless for studying the morphology of the organisms. (2) Colonies of trypanosomes stain deeply and only a blurred outline of the whole mass is seen. (3) Romanowsky's stain, and similar stains, deposit masses of precipitate and fail to bring out any detail in the organisms. By the use of the "Borrel-blue" stain, introduced by Laveran and Mesnil for staining trypanosomes in blood-films, beautiful preparations may be obtained, which show clearly the structure and detail of the colonies.

"Borrel-blue" is made by adding a small quantity of freshly precipitated silver oxide to a saturated watery solution of Methylene-Blue (Höchst). The following directions for staining are given by Laveran and Mesnil ('01, p. 680). (1) Make a thin film and place it, as soon as it is dry, in absolute alcohol for 5 minutes. (2) Transfer the film, without drying, to the stain which is composed of:

Borrel-blue	1 part.
1 $^0/_0$ Eosine (Höchst)	4 parts.
Distilled water	6 parts.

The film must be placed in the stain the moment the latter is mixed. (3) After staining

trypanosomes and colonies, which can only be seen in stained preparations, remain to be described.

(1) *Protoplasm.* The protoplasm of the cell is usually homogeneous in structure. It stains a pale blue, or, if the preparation has been stained for a long time, a pale violet. Small, deeply stained, granules of a red or violet colour are sometimes seen but they are seldom numerous. Vacuolisation of the protoplasm is rare, but occasionally a large highly refractile vacuole, whose diameter is nearly equal to the width of the trypanosome, is found. [These vacuoles have a greenish tint when seen in the living parasite.]

(2) *Nucleus.* The position of the nucleus is variable. In young pear-shaped forms it is usually placed at one side of the cell in the anterior half of the body of the trypanosome. In the longer spindle-shaped forms the nucleus is more centrally placed. The nucleus is round or oval in shape, and stains a light purple-red colour.

(3) *The Centrosome.* An intimate connection appears to exist between the nucleus and the centrosome. In young forms these structures are always found close to one another, in some cases the centrosome appears to be embedded in the peripheral portion of the nucleus, from which however it is easily distinguished by its staining deeply. The centrosome is usually placed either at one side of the nucleus or at a variable distance anteriorly to it. In free forms, at any rate, I have never observed it lying posteriorly to the nucleus. The centrosome is most commonly seen as an elongated, rod-like, structure, with sharply defined edges, lying transversely to the long axis of the trypanosome. Sometimes it has a crescentic outline and occasionally is represented by two small round bodies, placed in close apposition.

the film for 15 minutes, wash it well in water. (4) Place the film in 5 % tannic acid solution for 10 minutes. (5) Wash the film again in water and dry it; if a precipitate is deposited on the film, clear in clove oil and then wash it in Xylol. (6) Mount the preparation in Canada Balsam.

On account of the dense precipitate which is deposited, I found it advisable to place the coverslips in the stain with their film-surface downwards. Any precipitate, adhering to a film, may be easily removed by immersing the coverslip in water and brushing it lightly with a camel's hair brush.

When this method of fixing and staining is used, the outlines of the organisms are well preserved. The protoplasm stains a pale blue, the nucleus a bright reddish-violet colour. The flagellum stains either a bright red or the same colour as the nucleus. By prolonged staining (12–24 hours), excellent preparations are obtained for studying the outline and structure of the Trypanosomes in colonies. The coloration is however somewhat different and is less intense (see Fig. 1, Plate IV. where the protoplasm of the cells has a pale violet tint).

The centrosome is apparently homogeneous in structure, it stains a deep violet or crimson colour.

(4) *Flagellum.* In well stained preparations nearly every trypanosome shows a flagellum, which arises from the centrosome. The flagellum takes a straight or slightly sinuous course and passes, apparently, through the protoplasm of the cell. The free portion of the flagellum is often two, three, or four times the length of the body of the trypanosome. The centrosome is sometimes situated so near to the anterior end of the body of the trypanosome that the flagellum is almost entirely extracellular. The flagellum stains a bright red colour.

(5) *Undulating membrane.* It is doubtful whether this structure exists in the cultural forms of *Tr. Lewisi.* If present the undulating membrane must be exceedingly small, for the flagellum projects from the body of the trypanosome as a free structure for almost its entire length.

(6) *Colonies.* There is little to add to the description, given above, of the structure of the trypanosomes and of their arrangement in the colonies. Fig. 2, Plate IV., from a drawing made of a stained preparation of a colony, which has been much flattened out, shows that the trypanosomes sometimes possess very long flagella. The bunching together of these structures in the centre of the colony is better shown in the other illustrations. McNeal and Novy, in their description of cultures of *Tr. Lewisi*, call these masses of trypanosomes "rosettes," but in their paper on the cultivation of *Tr. Brucei* they also use the term "colony." The latter term seems to me to be more suitable for the following reasons:—(1) Among the Protozoa colony-formation is not infrequent and results from the incomplete separation of daughter-cells from their mother-cells. (2) A second reason for using the word "colony" instead of "rosette" is that the latter term has been already applied to the groups of multiplication forms found in rats during the early stages of infection. In Fig. 4, Plate IV., is given a drawing of one of these rosettes for the purpose of comparison.

The trypanosomes contained in the rosette are similar in shape to many of the organisms found in colonies in cultures of *Tr. Lewisi*, but they are attached at their posterior extremities and their flagella radiate outwards.

Novy and McNeal (I. '04, p. 28) have not apparently succeeded in staining the flagella in their preparations, though they noted the position of the centrosome. They expressed the opinion that the end of the trypanosome, pointing towards the

periphery of the colony, was the anterior extremity and that from it a flagellum would arise if the cultural conditions were perfected.

Measurements of the cultural forms of Tr. Lewisi.

Pear-shaped forms:

(1) Body. 3·6—4·4μ long and nearly as broad.

(2) Flagellum. Two to four times the length of the body.

Spindle-shaped forms. 14—16 × 2·4—3·5μ (flagellum not included).

Smaller and larger forms are frequently found. Novy and McNeal (I. '04, p. 26) give measurements varying from 2—60μ. The adult, parasitic form of *Tr. Lewisi* measures 24—25 × 1·5μ (Laveran and Mesnil, 1901, p. 681).

Mode of multiplication in cultures of Tr. Lewisi.

The mode of multiplication of these parasites in the rat has been already described (p. 28). The trypanosomes appear to multiply in cultures in a similar manner. In a young culture, free forms, dividing longitudinally, are common, but, as a rule, the mother-cell cannot be distinguished by its greater size. In stained preparations, forms which are undergoing *segmentation* are occasionally seen; the cells contain numerous nuclei and centrosomes from which arise flagella, and the protoplasm is incompletely segmented. Some of the trypanosomes found in cultures are exceedingly minute, small enough to pass through a Berkefeld filter (Novy and McNeal, I. '04, p. 28).

Degeneration of the cultural forms of Tr. Lewisi.

The faint shadowy forms of degenerated Trypanosomes remain practically unstained, with the exception of their centrosomes and flagella. Usually the outline of the cell is indicated faintly by dots of stain of a pinkish colour. Flagella, which have become detached from their trypanosomes, are frequently seen in cultures. They are found lying either singly or in clusters. The centrosome is often found still to be attached to the flagellum. Similar objects have been described in the blood of infected rats by Laveran and Mesnil.

Summary of the chief differences in morphology between the cultural and the parasitic (adult) forms of Tr. Lewisi.

Cultural forms.	*Parasitic forms.*
Exceedingly active.	Very active.
Very variable in size and shape, generally spindle-shaped or pear-shaped.	Size only varies within narrow limits. Body is slightly fusiform and has sharp pointed extremities.
Nucleus is variable in position.	Nucleus is invariably situated at the middle of the anterior half of the body of the parasite.
Centrosome is found either close to the nucleus or at a variable distance anteriorly to it. It is usually elongated.	Centrosome is found at a short distance from the posterior extremity and is usually round.
Undulating membrane is not developed.	Undulating membrane is well developed and is usually thrown into one or two folds.
Flagellum is frequently very long. Its basal portion is very short owing to the position of the centrosome.	Flagellum is much shorter, relatively to the length of the body of the parasite. If traced backwards in the free border of the undulating membrane, the flagellum is seen to arise from the centrosome.

II. *Cultivation of Tr. Brucei.*

Tr. Brucei can be cultivated on blood-agar in the same way as *Tr. Lewisi* but with much greater difficulty. Novy and McNeal (2. I. '04, p. 6) using the blood of different animals, infected with Nagana, for inoculating the media, failed to secure cultures twenty-five times in succession. In another series of experiments they were successful in four out of twenty-five attempts at cultivation. Subcultivation proved to be a more certain and rapid process. In their preliminary notice Novy and McNeal stated that one of their cultures was in its eighth generation, the period of cultivation having extended over a hundred days. In a later paper (V. '04, p. 3) these authors state that one of their cultures, started on August 27th, 1903, is now in its twenty-seventh generation. No mention is made of the virulence of the culture.

There are differences between *Tr. Lewisi* and *Tr. Brucei* which may account for the difficulty in cultivating the latter. Nagana blood, kept *in vitro*, rapidly loses its infectivity. Kanthack, Durham and Blandford (X. '98, p. 117) state that the parasites sometimes become motionless within twenty minutes from the time of collecting the blood, but generally motile forms are found for two or three days and occasionally for five or six days. It was exceptional to find that the blood was

infective at the end of three or four days. The rat trypanosome, on the other hand, may be maintained alive for more than fifty days, as shown by Laveran and Mesnil (1901, p. 679), if the blood is collected aseptically and kept cool in an ice-chest. The rapid alteration of the Nagana trypanosomes on change of environment, probably accounts for the difficulty in obtaining cultures of these organisms. In blood-agar, the life of the Nagana trypanosome is greatly prolonged, although there may be no signs of multiplication of the parasite. I have found involution forms, possessing active flagella, three weeks after adding the infected blood to the medium.

It is well to note the most favourable conditions for cultivating *Tr. Brucei.* The quantity of blood added should be at least equal in volume to the agar which is used. On account of the slow rate of multiplication of the trypanosomes and the rapidity with which cultures degenerate, the latter are best incubated at a temperature of 25° C. (see incubation of cultures of *Tr. Lewisi*, p. 29).

Three out of ten attempts which I made to cultivate *Tr. Brucei*[1] were successful. Two of the cultures were obtained from white rats and the third culture was made from a rabbit. The unsuccessful attempts were made with rats (2), mice (2), rabbits (3). Rabbits are not suitable animals from which to obtain cultures because their blood contains few parasites. Mice are inconveniently small to work with. I think that if I had confined my cultural experiments to rats there would have been a smaller percentage of failures.

Generation 1. Incubated at 25° C. Made on March 25th, 1904. Two loopfuls of the blood of a white rat were added to a tube of rabbit-blood-agar. The rat, whose blood was swarming with parasites, had been injected intra-peritoneally four days previously with blood taken from a Nagana rabbit. A drop of the culture, examined 24 hours later, was found to contain two or three active trypanosomes per field. On the fourth day several clumps of trypanosomes, containing as many as twenty or thirty trypanosomes, were found. The trypanosomes were irregularly arranged but possessed very active movements; they were somewhat granular in appearance. Pairs of trypanosomes, linked by their posterior extremities, were common. Free actively motile forms

[1] The strain of *Nagana*, possessed by this Laboratory, is well known. It was procured in 1896 from S. Africa by the Royal Society for investigations, which were made by Kanthack, Durham and Blandford ('98, p. 100), and has since then been maintained by the infection of rabbits. This strain was used by Laveran and Mesnil in their researches and by Novy and McNeal in their cultural experiments.

were not numerous. On the fifth day some of the trypanosomes were observed to contain vacuoles, which were very refractile in appearance and of a greenish tint. The vacuoles varied in size and number, they were usually situated in the anterior half of the organism. This culture never contained many colonies or free forms. At the end of three weeks the organisms were very granular in appearance and their movements were sluggish. On the twenty-eighth day, repeated attempts at subcultivation having in the meantime failed, 2 c.c. of fresh, defibrinated normal rabbit's blood were added to the culture, and 1 c.c. of this mixture was then added to a tube of freshly prepared rabbit-blood-agar, in order to obtain generation 2.

Generation 2. This culture (generation 2) was examined daily for eleven days, without finding any evidence of growth, but on making an examination on the twenty-fifth day, the culture was found to be swarming with organisms. Some of the colonies were of large size, containing about a hundred trypanosomes. This culture contained a few granular, motile forms on the thirty-ninth day.

Generation 3. Made with three loopfuls of culture of generation 2 on the twenty-sixth day. No living trypanosomes were found for the first three days, but on the fourth day a drop of the culture contained two small *rosettes* and several free forms. On the seventh day rosettes and free forms were numerous. The rosettes, which seldom contained more than twelve trypanosomes, were perfectly symmetrical in appearance, the flagella being directed outwards. The rosettes occasionally moved slowly across the field, but as a rule the active, lashing, movements of the individual organisms, composing the rosette, merely imparted to the latter a slight swaying movement. The rosettes were generally partly hidden by red blood corpuscles, which the trypanosomes seemed to attract around themselves. No rosettes were found on the eleventh day, but there were several large masses of trypanosomes which I shall describe subsequently as *colonies*. Free forms were also very numerous. On the fourteenth day there were few colonies, but trypanosomes, either free or in pairs, were numerous. This culture was alive on the twenty-first day, but the trypanosomes were not so numerous and many of them showed signs of degeneration. Three other subcultures, made at the same time as generation 3, from generation 2, were similar in character. Several attempts were made to subcultivate from generation 3, and from a parallel culture, but all failed[1].

[1] These subcultures were made just before taking a holiday; on my return in three weeks' time the original cultures were found to be dead.

Culture A. Made from the blood of a rabbit infected with Nagana. The blood of this rabbit was used for making the blood-agar as well as for inoculating the cultures with trypanosomes. The parasites were apparently all dead at the end of fourteen days, but, on examining the tube a month later, numerous dead clumps of trypanosomes and a few granular, slightly motile, colonies and free forms were found. Subcultures failed.

Culture B 1. Made from a white rat. A moderate growth was obtained at the end of three weeks. The culture showed signs of degeneration at the end of a month, and was quite dead in six weeks' time.

Culture B 2. Made from culture B 1, on its twenty-fourth day. A good growth was obtained at the end of three weeks, but unfortunately the culture became contaminated and further subcultivation was impossible.

Infection experiments with cultures of Tr. Brucei. Novy and McNeal (I. '04, p. 19) state that the virulence of the culture depends upon the temperature of cultivation, duration of growth, and possibly on the composition of the medium. They found that the exposure of a culture to a temperature of 34° C. for 2—6 days rendered the culture non-virulent, except in two instances (rats died on 14th day after injection with cultures incubated at 34° C. for five days). Most of their cultures, incubated at room temperature or at 25° C., were found to be virulent. The inoculations were made with cultures which varied in age from 7 to 22 days.

All the cultures of Nagana, which I have tested, have failed to produce infection in animals. Probably the cultures were too old; possibly in the case of Mouse no. 2 (see below) the dose was too small. The animals were injected intra-peritoneally with the culture fluid, which was mixed with a small quantity of normal salt solution. A list of these experiments is given below.

No. of animal	No. of generation	Amount injected	Age of culture	Result
Mouse 1	2	1 loopful	32 days	non-virulent
,, 2	3	1 loopful	15 ,,	,,
,, 3	B 1	3 loopfuls	25 ,,	,,
Rat 1	B 2	1 c.c.	28 ,,	,,

Morphology of Tr. Brucei in cultures.

(A) *Unstained preparations.* The organisms found in cultures of *Tr. Brucei* do not vary greatly in size and shape, and they resemble

closely the forms found in the blood. If a young culture is examined, the trypanosomes are found to possess very active movements. Sometimes they advance across the field moderately quickly, but their rate of movement is always much slower than that of the rat trypanosomes, whose flagella are longer and more rapid in action.

The undulating membrane is well-developed and its wave-like contractions are very clearly seen. The cultural forms are smaller than the trypanosomes found in the blood.

(B) *Stained preparations.* The organisms stain similarly to *Tr. Lewisi* but more deeply.

(1) *Body of the trypanosome.* This is usually bent or curved, its anterior extremity is pointed, the posterior end is usually slightly blunter. The protoplasm invariably contains a few deeply stained granules of a red or violet colour. The vacuoles, which were described on p. 39, are seen as clear circular spaces with sharply defined outlines in stained preparations. (See Figs. 5 and 6, Plate IV., and Figs. 3 and 4, Plate V.)

(2) *Nucleus.* The nucleus is round or oval in shape, and in older forms it breaks up into masses of chromatin, which are found distributed throughout the protoplasm of the cell.

(3) *Centrosome.* This structure is much smaller than in *Tr. Lewisi*, it is usually circular, but sometimes it is elongated. The centrosome stains a deep red or purple colour, it is sometimes difficult to distinguish it from the other granules. It is generally found close to a vacuole, sometimes it lies close to the nucleus, but it is nearly always *posterior* to the latter structure. (See *Tr. Lewisi*, p. 34.)

(4) *Flagellum.* The origin of this structure from the centrosome cannot always be seen. The flagellum takes a tortuous course along the free border of the undulating membrane, and projects for a short distance from the anterior extremity.

(5) *Colonies.* A description of the symmetrical "*rosettes*" has been already given on p. 39; it is more difficult to describe the larger masses of trypanosomes, composing the *colonies.* In the *living state* these structures are composed of closely packed, somewhat irregularly arranged, writhing masses of trypanosomes. It is rare to find colonies of a large size. The larger ones are not circular in outline as are colonies of *Tr. Lewisi*, but are generally elongated. Most of the flagella are directed in an outward direction. The active movements of the trypanosomes and the large glistening vacuoles, with which they are studded, give these colonies a singularly beautiful appearance.

It is not easy to obtain stained preparations in which the rosettes and colonies retain their form, since separation or massing together of the trypanosomes usually occurs in the process of making a film. Figs. 5 and 6, Plate IV. are from drawings of two rosettes (in stained films), which have retained their symmetry. Photographs of the same are given in Figs. 3 and 4, Plate V., for the purpose of comparison.

Measurements of the cultural forms of Tr. Brucei.

The following measurements were taken from stained preparations:—

Average dimensions (flagella included) } 18—23μ by 2·5—3·5μ (*26—27 by 1·5—2·5*μ).
Length of the flagella (free portion) 3—5μ.
Diameter of the vacuoles up to 1—2μ.

The dimensions printed in italics are those given by Laveran and Mesnil (1902, p. 19) for *Tr. Brucei* in the blood of rats.

Degeneration of the cultural forms of Tr. Brucei. The presence of numerous, large, highly refractile "globules" in the cultural forms of *Tr. Brucei* is attributed by Novy and McNeal to degeneration of the organisms owing to imperfections of the culture-medium. These globules become more numerous as the age of the culture advances. I have kept trypanosomes under observation in hanging drops for several hours without observing any alteration in the position or shape of their globules. The latter resist staining completely. Laveran and Mesnil (1904, p. 21) suggest that the globules, described by Novy and NcNeal, are of the same nature as the refringent, unstainable, granules found in *Tr. rotatorium*, and in *Tr. Lewisi*, if the latter be injected into the peritoneal cavity of a guinea-pig. The same authors attribute the vacuolation of *Tr. ugandense* (Castellani, 1903) to imperfect fixation of the trypanosomes, such as occurs in films made of cerebro-spinal fluid.

Mode of multiplication of Tr. Brucei. All observers agree that multiplication of this parasite occurs by longitudinal division. The process is similar to that occurring in *Tr. Lewisi*, but the division results in the formation of trypanosomes, which are approximately equal in size, and the flagellum splits along the whole or greater part of its length. The cultural forms multiply in the same way, but division is often incomplete, which accounts for the presence of so many paired forms in young cultures. Incomplete separation of the dividing forms also undoubtedly leads to the formation of the rosettes. The arrangement of the trypanosomes, composing the rosettes, with their flagella

directed outwards, is similar to that in the multiplication rosettes, found in the blood of rats infected with *Tr. Lewisi.* The question naturally arises, is the mode of formation identical in each instance? I have searched numerous preparations made from cultures of *Tr. Brucei* without finding any forms undergoing segmentation, and therefore conclude that the cultural, like the parasitic, forms of *Tr. Brucei* do not multiply by the process of segmentation.

Morphology of the cultural forms of Tr. Lewisi and Tr. Brucei summarised and contrasted.

(A) *Unstained preparations.*

Tr. Lewisi.	*Tr. Brucei.*
(1) Spindle-shaped or pear-shaped. Very variable in size, usually measure 3—5μ or 14—16μ (excluding the flagellum). Smaller and larger forms frequently seen.	Resemble the forms found in the blood of infected animals but are shorter and more pointed. More constant in shape and size than *Tr. Lewisi*, measure 15—20μ (excluding the flagellum).
(2) Move with great rapidity, generally in straight lines. The body of trypanosome is not curved or bent.	Movements are much slower and are generally of a wriggling character.
(3) *Protoplasm* is clear and homogeneous, rarely it contains a large single vacuole.	Protoplasm soon becomes slightly granular, invariably contains two or three large vacuoles.
(4) *Flagellum* very long and active, often quite rigid except at the point where it issues from the anterior extremity of the trypanosome.	Comparatively short but very active.
(5) *Undulating membrane* absent, unless it is developed in a very minute form at the base of the flagellum.	Well developed; its contractions are described by Novy and McNeal (I. '04, p. 27) as passing round the cell in a "spiral" direction.
(6) *Colonies* form large masses of cells, which are symmetrically arranged with their anterior extremities directed centrally. Huge colonies, visible macroscopically as small whitish granules, are formed by the coalescence of several colonies.	Colonies are of small size and are much less numerous. The younger colonies may present a symmetrical rosette-like appearance, the flagella being directed outwards. In the older colonies, the trypanosomes are closely packed together but are somewhat irregularly arranged. Secondary massing together of the colonies does not occur to any extent.
(7) Trypanosomes multiply rapidly. Cultures *swarm* with colonies and free forms and remain alive for three months or longer. Cultures retain their *virulence* for a long time.	Trypanosomes are never so numerous and degenerate rapidly; the culture being generally dead at the end of two months. Cultures rapidly lose their virulence, the time taken to do so depending upon the temperature of incubation.

(B) *Stained preparations.*

(8) *Protoplasm* stains a pale blue.	Protoplasm stains a deeper blue, and frequently contains deeply stained granules.
(9) *Centrosome*, usually rod-shaped and situated either at the margin of the nucleus or just anteriorly to it.	Centrosome much smaller, round or elongated and often difficult to distinguish from the other granules. Generally situated at the posterior end of the trypanosome.
(10) *Flagellum*, long and thick and projects for almost its entire length from the cell. Measures two to four times the length of the body of the trypanosome.	Flagellum is short and fine and can be traced backwards along the free border of the undulating membrane to end in the centrosome. Measures 3—5μ.

Since the above was written Novy, McNeal, and Hare (v. '04, pp. 1—8) announce that they have succeeded in cultivating the *Surra Trypanosome* of the Philippines. They found that the culture differed from those of *Nagana* in the following important respects: (1) The trypanosomes were larger, their average length was 25—35 μ, the flagella were very long. (2) The trypanosomes were very actively motile and moved either *backwards* or *forwards.* (3) The protoplasm of the anterior portion of the cell contained a large number of small "*granules or globules*" about 0·3—0·5 μ in diameter, of a yellowish or greenish tint; in forms dividing longitudinally, these globules were arranged in parallel lines, one row in each half of the dividing parasites. (4) There was an entire absence of rosettes and colonies.

The cultures (three in number) failed to subcultivate or to infect animals, although they remained alive for forty-eight to sixty-five days. The above mentioned investigators conclude therefore that *Surra* and *Nagana* are *distinct* forms of Trypanosomiasis, and they point out that Laveran and Mesnil and Nocard, as the result of immunising experiments, have expressed the same opinion.

Summary.

Tr. Lewisi has been cultivated by me for nine generations, the duration of cultivation extending over a period of nine months. The culture is still alive and is now in its tenth generation. A culture of *Tr. Brucei* was cultivated through three generations for a period slightly exceeding eighty days. These cultural experiments, however, in no way compare, in duration of time, with those of Novy and McNeal, who have cultivated *Tr. Lewisi* through twenty-six generations in two years, and *Tr. Brucei* through twenty-seven generations in eight months. Nagana trypanosomes degenerate rapidly in cultures and then become non-virulent, but a culture of *Tr. Lewisi* retains its infectivity

for rats for a long period. From the study of well stained preparations it has been found that the cultural forms of the rat trypanosome differ considerably from those of the adult parasite found in the blood. The chief points of difference are: (1) The trypanosome is exceedingly motile and is generally spindle-shaped. (2) The centrosome is placed anteriorly. (3) The flagellum is very long and active and has but a short intracellular course, in consequence of the position of the centrosome, from which it arises. (4) No undulating membrane is apparent. (5) The trypanosomes form colonies, which ultimately contain many thousands of individuals. The flagella of the latter are directed *centrally*. In rats, infected with cultures of *Tr. Lewisi*, the usual forms of parasite appear. The cultural forms of *Tr. Brucei* resemble the trypanosomes found in the blood, but are more active. The chief points of difference, viz. development of two or more vacuoles and numerous granules and sometimes the breaking up of the chromatin of the nucleus, are probably due to degeneration. The colonies, found in cultures of *Tr. Brucei*, consist of a comparatively small number of trypanosomes, which are arranged irregularly or with their flagella directed *peripherally*. The differences between cultures of *Tr. Lewisi* and *Tr. Brucei* are summarised on page 43.

In conclusion, I should like to acknowledge my indebtedness to Dr G. H. F. Nuttall, F.R.S., for his suggestion that I should repeat some of the interesting researches of Novy and McNeal. Dr Nuttall has very kindly examined many of my preparations, and he has placed at my disposal his very complete collection of published papers on the subject of Trypanosomiasis.

To Professor Sims Woodhead I am indebted for the many advantages which I have gained by working in the Pathological Laboratory, Cambridge.

The photographs were taken in this laboratory by Mr W. Mitchell, who has spared neither time nor trouble in his endeavours to obtain photographs suitable for reproduction. The various shades of red and blue, with which the organisms were stained, added considerably to the difficulties of micro-photography.

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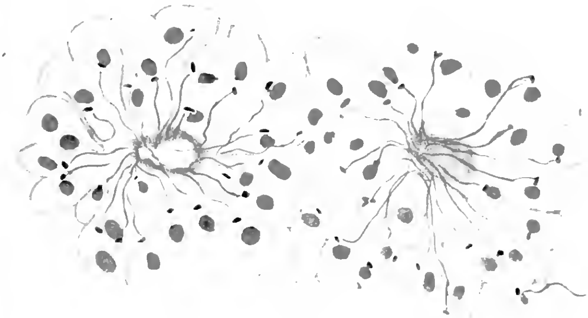

Fig. 1.

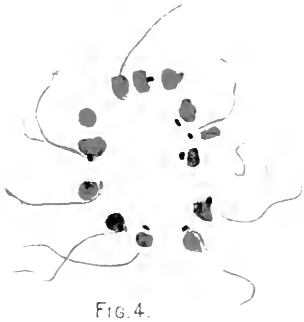

Fig. 4.

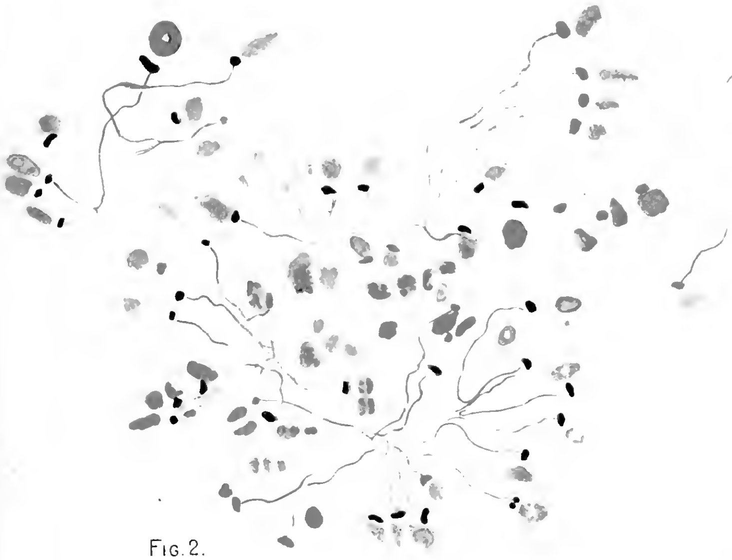

Fig. 2.

Fig 3

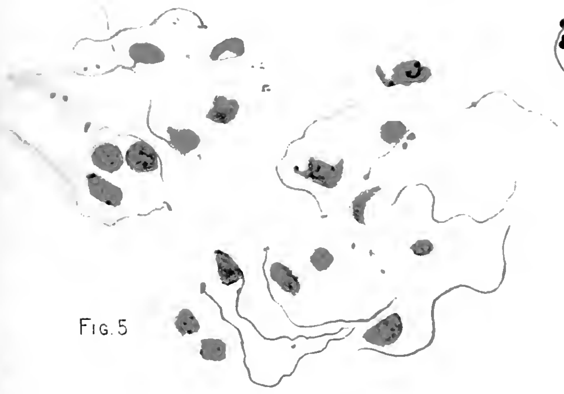

Fig. 5

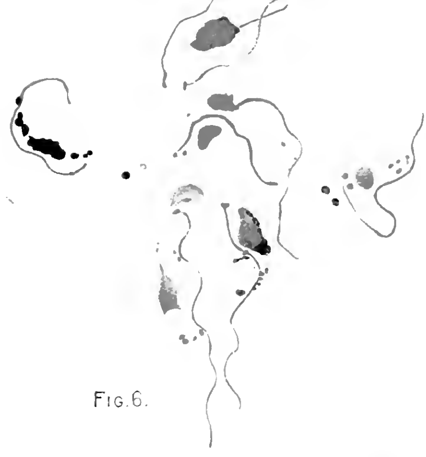

Fig. 6.

R.D Smedley, del.

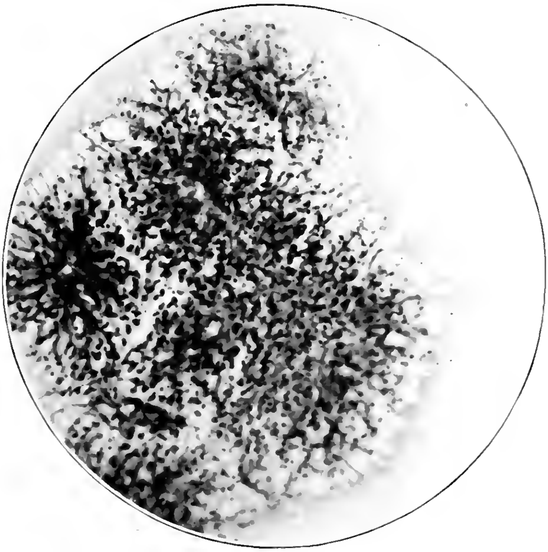

Fig. 2.

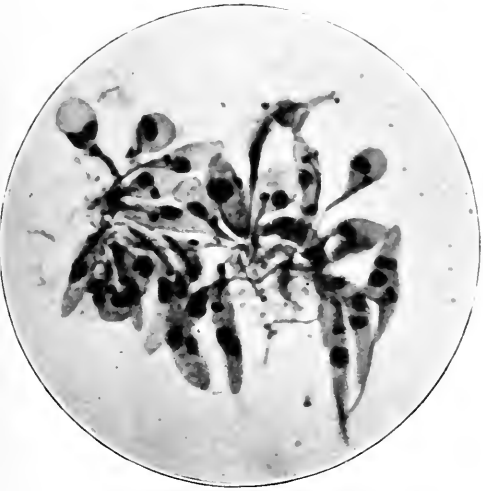

Fig. 1.

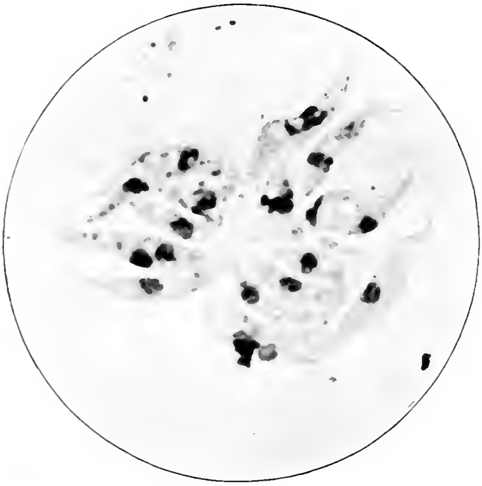

Fig. 3.

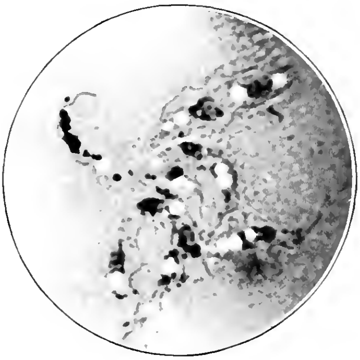

Fig. 4.

EXPLANATION OF PLATES IV AND V.

Tr. Lewisi.

(From film-preparations stained by the method described on p. 33.)

Plate IV.

Fig. 1. Colony from a culture, 40 days old, of 2nd generation. ×1900 approx.

Fig. 2. Colony, much flattened out, from the same culture as above. ×1900 approx.

Fig. 3. Rosette, forming by process of segmentation. Blood-film preparation from a white rat, inoculated four days previously with a culture of *Tr. Lewisi.* ×1900 approx.

Fig. 4. Another rosette, more advanced stage of development. From the same preparation as that described under Fig. 3. ×1900.

Plate V.

Fig. 1. Young colony. From a culture, 25 days old, of 2nd generation. ×1250.

Fig. 2. Large colony. From the same preparation as that described under Plate I., Fig. 1. ×800.

Tr. Brucei.

Plate IV.

Fig. 5. Rosette, from culture, 8 days old, of 3rd generation. ×1900.

Fig. 6. Rosette, from a culture, 9 days old, of 3rd generation. Period of cultivation extended over 62 days. ×1500.

Plate V.

Fig. 3. Same rosette as that described under Plate IV., Fig. 5. ×1250.

Fig. 4. Same rosette as that described under Plate IV., Fig. 6. ×1000.

ON THE EPIDEMIOLOGY OF PLAGUE.

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Characters of areas in which plague is endemic.

In areas in which plague is present, or has recently been present, in India, as an epidemic, no definite relation has been observed between intensity of plague (apart from its persistence), and badness of sanitary condition of dwellings[1]. On the other hand, in areas in which plague is present endemically, so far as evidence goes, very unsatisfactory sanitary conditions exist. Such areas are generally situated in mountainous countries (Garhwal, Yunnan, Beni-Cheir, Transbaikalia). The inhabitants of such places, owing to the difficulty of obtaining water for domestic purposes, are apt to be filthy both in their houses and persons. In Garhwal, Yunnan, and Beni-Cheir

[1] In Bombay only 8·6 per cent. of tenements condemned as unfit for human habitation in 1897 were situated in wards F and G, which were the portions of the town most severely attacked by plague. For other facts of the same nature see Hankin, "La propagation de la Peste" (*Annales de l'Institut Pasteur*, November, 1898, p. 705). Dr Weir, the Health Officer of Bombay in 1896, stated that the houses in the suburban villages near Bombay which suffered severely from plague were not so overcrowded as the houses in the slums of the city which were far less severely attacked. (*Evidence before the Indian Plague Commission*, Vol. III. p. 311.) Mr Winter, who was in charge of the plague operations in Jawalapur, stated that more cases of plague occurred in large well-built houses than in mud huts, at any rate at the commencement of the outbreak. In a later paragraph it will be pointed out that a large proportion of the earlier cases in Jawalapur were of grain dealers, that is to say of persons who, though well-to-do and living usually in well-built houses, were likely to come into places infested with rats. (*Evidence before the Indian Plague Commission*, Vol. II. p. 52.)

domesticated animals are stabled in the houses, the paucity of level area making separate provision for cattle a matter of difficulty. Stable refuse consequently is liable to accumulate in the lower floors of the houses, producing conditions favourable to the presence of swarms of fleas.

As illustrating the above statement, the following description of an infected house in the endemic area of Garhwal is quoted from Planck[1]:

"The infected house was found to be an old, roughly-built, thatched double tenement, placed on a solitary ledge. The site generally, filthy from manure and overgrown with excessive vegetation. The lower rooms had long been used as cattle or goat pens, the upper had apparently never been cleansed or washed since the house was built, and were infested with hungry fleas in such extraordinary numbers that a few minutes' stay on the premises necessitated an immediate resort to a neighbouring stream, there to await the arrival of a change of clothing. The two native officials who assisted at this inspection were attacked in like manner, and a few minutes after the house had been hastily fired all three persons were immersed in the pool below it."

In the Mesopotamian area in which plague appears to be endemic, I am informed by Mr Syad Abulhasun, Tehsildar of Agra, who has travelled in that country, that the villages are infested with large numbers of fleas. He states that though the inhabitants are ignorant and uncivilised they instantly vacate and burn down their villages on the appearance of plague.

Up to the present time, endemic areas have been excluded from any great amount of human traffic or intercourse, either owing to the paucity of their inhabitants or to the nature of the country. Probably this is the reason why they have not, more often than has been the case, served as sources of outbreaks of plague. But we cannot assert that this state of things will continue to exist in the future. The Trans-Siberian Railway may open up the Transbaikalian and Mongolian endemic areas; the Cape to Cairo Railway that of Uganda. A projected railway from Burmah to China might, if carried out, give further opportunities of acquaintance with the endemic plague of Yunnan, and a proposed railway along the Euphrates valley may do the same for the infected area of Mesopotamia. Thus plague, far from being a disease that promises to become extinct, threatens to be an increased source of anxiety.

[1] *Annual Report* of the Sanitary Commissioner for the North-West Provinces and Oudh for the year 1876.

Persistence of plague in endemic areas.

A study of the plague in the Indian endemic area[1] of Garhwal indicates that it does not persist there owing to a constant succession of cases of the disease. On the contrary the disease remains apparently extinct for years and then suddenly breaks out in fully virulent form. The observations of Planck[2] leave little room for doubt that during the intervals between successive outbreaks the microbe does not continue to exist owing to a succession of passages either through human beings or through rats. Judging from present evidence it would appear that it exists in a latent state so far as these particular living beings are concerned.

Indian plague outbreaks and wanderings of fakirs.

The following table[3] gives a complete list of recorded plague outbreaks in India proper, and also of outbreaks in the endemic area of Garhwal, including Kumaon. Garhwal is a mountainous and somewhat inaccessible country, having but little ordinary traffic with the rest of India. Only on one occasion (namely, a small outbreak at Moradabad in 1853) is it likely that this ordinary traffic has resulted in an exportation of the disease. Two sacred shrines are present that are annually visited by pilgrims from the plains of India, but no case is known of these pilgrims having been concerned in the transmission of the disease. But there are in Garhwal numbers of strict ascetics or fakirs who usually reside at or near the sacred shrines. On certain occasions these fakirs travel through Garhwal and the plains of India to places of pilgrimage where from time immemorial festivals have been held at twelve-yearly intervals. It was originally suggested by the German Bombay Plague Commission that fakirs from Garhwal were the source of the Bombay plague. The facts included in the following table strongly suggest that most other known outbreaks of Indian plague have a similar origin.

[1] The phrase "endemic area" will, I hope, be regarded as an allowable contraction for the phrase "area in which the disease is endemic." Convenience may justify a use of the word not implied by its derivation.

[2] *loc. cit.*

[3] For facts included in this table I am chiefly indebted to "The Plague in India" (*Official Report*, compiled by R. Nathan, Indian Civil Service), and to Hutcheson, "Mahamari" (*Transactions* of the Indian Medical Congress held at Calcutta in December 1894, p. 304).

Date	Pilgrim festivals visited by Garhwali fakirs, held at twelve-yearly intervals at :—	Plague in endemic area of Garhwal including Kumaon	Plague in other parts of India
1344	Nassik ...	... ...	Army of Sultan Mahommed Tughlak destroyed by pestilence probably near Deogiri a town at a short distance from Nassik.
1608	Nassik		
1611	...	... ...	Plague said to have commenced in Punjab. Lasted 7 years, and spread to Delhi, Agra, Cashmere, and Kandahar.
1680	Nassik		
1683	...	... ...	Confined to Western India. Lasted in Ahmedabad for 8 years.
1812	Nassik ...	... ...	Began in Gujerat and lasted 9 years.
1822	Allahabad	First recorded appearance of plague in Garhwal	
1823		A few villages attacked	
1824	Nassik	0	
1825	0	0	
1826	0	0	
1827	0	0	
1828	0	0	
1829	0	0	
1830	0	0	
1831	0	0	
1832	0	0	
1833	0	0	
1834	Allahabad	Severe	
1835	0	Severe	
1836	Nassik	Severe	The Pali Plague commenced at village of Taiwali near Pali. Stated by villagers to have been brought by wandering fakirs. Lasted 2 years.
1837	0	Severe in one district	
1838	0	0	
1839	0	0	
1840	0	0	
1841	0	0	
1842	0	0	
1843	0	0	
1844	0	0	
1845	0	0	
1846	Allahabad	At source of Ramgunga and nearly depopulated Sarkot	
1847	0	One village	

Date	Pilgrim festivals visited by Garhwali fakirs, held at twelve-yearly intervals at :—	Plague in endemic area of Garhwal including Kumaon	Plague in other parts of India
1848	Nassik	A few villages	
1849	0	Virulent in 2 villages	
1850	0	One village	
1851	0	Several villages	
1852	0	Some villages	
1853	0	0	In Moradabad, said to have been brought from Garhwal.
1854	0	0	
1855	0	0	
1856	0	0	
1857	0	0	
1858	Allahabad	0	
1859	0	Began in northern districts	
1860	Nassik	Severe	
1861	0	0	
1862	0	0	
1863	0	0	
1864	0	0	
1865	0	0	
1866	0	0	
1867	0	0	
1868	0	0	
1869	0	0	
1870	Allahabad	One village	
1871	0	0	
1872	Nassik	0	
1873	0	0	
1874	0	0	
1875	0	6 cases	
1876	0	327 deaths	
1877	0	535 deaths	
1878	0	10 deaths	
1879	0	0	
1880	0	0	
1881	0	0	
1882	Allahabad	0	
1883	0	0	
1884	Nassik	Severe	
1885	0	?	
1886	0	"A great number of deaths"	
1887	0	82 deaths	
1888	0	About 36 deaths	
1889	0	0	
1890	0	0	
1891	0	Six deaths	
1892	0	0	
1893	0	Two villages, 16 attacks	

Date	Pilgrim festivals visited by Garhwali fakirs, held at twelve-yearly intervals at:—	Plague in endemic area of Garhwal including Kumaon	Plague in other parts of India
1894	Allahabad	Probably two villages	
1895	0	0	
1896	Nassik	0	Plague commenced in Bombay.
1897	0	One village, 17 attacks	
1898	0	0	
1899	0	0	
1900	0	0	

(*Note.* According to Hutcheson the history of the disease in Garhwal from 1887 up to 1894 is imperfect, owing to the view having been adopted that it was identical with typhus fever.)

Plague often carried by persons not themselves infected at the time.

In view of the evidence now accumulated from the history of the Bombay outbreak, there can be no doubt that plague is, not infrequently, carried from place to place by persons who themselves escape the disease or who are not the first attacked in the places to which they have carried the infection. This curious fact is however not simply a feature of the Bombay plague. It was noticed both in the plague of Justinian and during the Black Death. Consequently the suggestion that Garhwali fakirs have been concerned with the spread of Indian plague does not imply that the fakirs themselves must in so doing have suffered from the disease.

Derivation of Bombay plague, probably Garhwal.

The question of the origin of the Bombay plague must now be considered. The first cases of disease recognised as plague occurred on the 10th of August, 1896. As to the source of this infection nothing was definitely known at the time. It was first supposed that plague was brought from Hong Kong, but no evidence exists that dock labourers or travellers from Hong Kong were among the first affected. So far as the evidence goes, those first attacked were principally grain dealers and the rats that haunted their shops, but not the rats that found a living in the docks. As already mentioned an alternative suggestion was put forward in the report of the German Bombay Plague Commission to the effect that the plague was imported into Bombay by fakirs from Garhwal.

The known facts bearing on the case are as follows: In July and August, 1896, about two thousand fakirs came to Bombay from northern India on their way to a religious festival at Nassik. They encamped for the most part in and near the temple compound of Walkeshwar in Bombay. But during the day they spent their time begging among the Bunnias (grain dealers) and Bhattias, in Mandvie, the district of the city where the plague first appeared. The attention of the Commissioner of Police was drawn to these fakirs owing to the primeval simplicity of their costume. They hotly protested against interference, saying that they always wore the same clothing in the Himalaya Mountains from whence they had come. On being asked in what part of the Himalayas they lived they replied Garhwal. At least four of them gave this reply. They somewhat outstayed their welcome, and during August the Bunnias of Mandvie combined together and paid the railway fares of 800 of them who had not previously started, to Nassik, where the festival was to commence on the 13th of that month. At its commencement the outbreak of plague, that began about this time, was almost wholly confined to the Bhattia and Bunnia communities who had been especially in contact with the Garhwali fakirs. In the week ending the 25th August three suspicious deaths from fever and pneumonia occurred in Walkeshwar where the fakirs resided. Of these one was a servant and two were "mendicants." These reasons for suspecting the fakirs to have imported the infection are to be found in the Report of the German Plague Commission.

Probable similar origin for earlier Indian outbreaks.

Another consideration, it appears to me, can be put forward bearing on the question. The origin of the outbreaks of plague that occurred in western India, in the years 1812 and 1836, needs to be explained as much as the origin of the Bombay outbreak of 1896. On the two former occasions there was no plague in Hong Kong. Neither did these Indian outbreaks commence in sea-ports, which had it been the case might have been a ground for suspecting an importation from abroad. Can these two former outbreaks have been due to the wanderings of fakirs? The Garhwali fakirs only visit western India on the occasion of the Nassik festival, which is held at twelve-yearly intervals. Consequently if the former Indian outbreaks of plague had been caused by Garhwali fakirs, they should have occurred in years in which the Nassik festival was held. The year 1896, the date of the Bombay plague, was such an occasion. Counting backwards, by twelve-yearly intervals, we find that the Nassik festival was held in 1836, the year of the Pali plague, and in the year 1812, the year of the Gujerat outbreak. It is at least a striking coincidence that of the eight twelve-yearly festivals held during the nineteenth century at Nassik, three should have coincided with outbreaks of plague, and these the only outbreaks that happened.

The Pali plague.

In the case of the Pali plague I have been so fortunate as to find some evidence connecting its origin with wandering fakirs. The disease is generally supposed to have commenced in the town of Pali in July 1836 among the calico printers, a caste numbering about 2000 persons, of whom 655 succumbed to the disease. But in the *Bombay Medical and Physical Society's Transactions for* 1839 (Vol. II., p. 1) is a report, by Forbes, an Assistant Surgeon, on the Pali plague. He states that there is no doubt that the disease originated in the village of Taiwali, "ten short koss" south-east of Pali, in the month of April, and that from thence it was brought into the town. According to the statements of the natives, a party of wandering fakirs (Gosains) "on their return from Dwarka in Kathiawar, halted near the village, close to some fields, and began to carry off dry thorns from the hedges to cook their food with; the Gosains fled, leaving their malediction on the men, who soon after sickened and died of this uncommon illness." In another publication[1] on the subject he says, "The most singular phenomenon remarked in connection with the breaking out of the disease, and adverted to in Mr White's report, was the death of all the rats of the village of Taiwali, during the latter half of April, and just before its first appearance. They lay dead in all places and directions, in the streets, houses, and hiding-places of the walls," and that "this death of the animal attended or preceded the disease in every town that was attacked in Marwar, so that the inhabitants of any house instantly quitted it on seeing a dead rat."

Thinking that the statement that the fakirs in Bombay had come from so distant a country as Garhwal needed some further confirmation, I made enquiries as to the habits of these fakirs from several inhabitants of Garhwal during a visit to Naini Tal in the Himalaya Mountains. I was also so fortunate as to meet a fakir who had been to Nassik from Garhwal on the occasion of the festival of 1896, as well as on previous occasions. He assured me that Garhwali fakirs never travel to the Bombay Presidency except on the occasion of these twelve-yearly Nassik festivals. They travel by different routes. His route, which I took down from his dictation, was as follows: Gangotri (in Garhwal), Hardwar, Gorakhpur, Patna, Rewa, Banda, Chitrakot, Jhansi, Indore, near the Nerbudda river, Bhusawal, Jalgaon, Dhoolia, Punchbhatti, Tirmook, Nassik. This measured on the map gives a distance of about 2,150 miles, which he had travelled in a little over five months, giving an average of about 14 miles a day. Other fakirs travelled, probably by more direct

[1] *Thesis on the Nature and History of Plague as observed in the North-Western Provinces of India* (published in Edinburgh in 1840 by Machlachan Stewart and Co.), p. 34.

routes, to Bombay, and returned thence by rail to Nassik. The statement that Garhwali fakirs only visit the Bombay Presidency on the occasion of the Nassik festivals is obviously of importance in this connection.

Characters of Bombay plague.

The plague in the Bombay Presidency differs from plague at present existing outside India in other important respects besides in its greater persistency and intensity. Firstly, it has shown its maximum virulence not in towns but in villages[1]. Secondly, though it spreads with facility from a town or village to a neighbouring village, it does not appear often to be carried to great distances in epidemic form. Further, in India, it shows no tendency to spread along trade routes as such.

With regard to the greater virulence of Indian plague in small towns and villages than in large towns, the cases of Bombay and Oporto may be cited and compared. In both cases there was panic among the inhabitants on the appearance of the disease, and in both cases the infection was carried into towns and villages in the neighbourhood. In the case of Bombay the outbreaks thus produced often exceeded greatly in virulence the original outbreak in that city. In the case of Oporto though isolated cases of plague occurred in many villages in the neighbourhood, in no case did the disease succeed in establishing itself in epidemic form outside the town. Similarly in Tamatave, the disease spread to the surrounding country, but appears to have died out there before it came to an end in the town itself. Generally it may be stated, that, so far as existing evidence goes, plague, in the present outbreak, outside India has attached itself especially to the crowded and insanitary portions of large towns.

Similar characters possessed by other Indian plagues.

Can we find among other outbreaks an analogy for the peculiar characters of the present Indian plague?

[1] The intensity of plague in towns and villages in the Bombay Presidency has been in inverse proportion to their size as exemplified by the following table, which relates to certain plague outbreaks occurring between 1897 and 1898 in that Presidency:—

Name of place	Number of inhabitants	Death rate from plague per 1000 inhabitants
Bombay	806,144	20·1
Poona	161,696	31·2
Karachi	97,009	24·1
Sholapur	61,564	35·0
Kale	4,431	104·9
Supne	2,068	102·5
Ibrampur	1,692	360·5

The death rate of Bombay for 1896–97 is derived from lower plague death rates from the crowded and less sanitary central portions of the city, and higher plague death rates from suburban districts where village conditions prevailed.

On referring to records of the plague outbreak in Western India in 1812, it appears that, so far as the evidence goes, it resembled the present Indian plague, both in its avoidance of trade routes as such, and in its great virulence in villages as compared with towns, during the first eight years of its existence[1]. It was only at about the end of this period that it showed itself as a virulent disease in a comparatively large town (Ahmedabad). Another plague having similar characters also occurred in Western India, namely, the Pali plague of 1836. Though this produced a high mortality in the small town of Pali, it appears to have been more destructive in the surrounding country, where in spite of the smallness of the area affected, it is variously estimated to have destroyed from 60,000 to 100,000 persons. Pali was then an important trade centre, yet the disease failed to spread along any trade routes from this town.

The fact that these three plagues in Western India have these characters in common harmonises with the suggestion that they have a common origin.

Resemblances of Black Death to Indian plague.

A more important outbreak that appears to have resembled the Bombay plague in its great intensity, its virulence in villages, and in other characters, is the Black Death, and to some extent the European outbreaks that immediately followed it[2]. According to Simon von Covino, who observed the Black Death in Paris, Montpellier, and probably other places, it was especially severe in the smaller towns (*suburbia*). Creighton[3] states that probably two-thirds of the country clergy were destroyed by this pestilence in England. He adds, "This alone would suffice to show that the virus of the Black Death permeated the soil everywhere, country and town alike. It is this universality of incidence that chiefly distinguishes the Black Death from the later outbreaks of plague, which were more often in towns than in villages or in scattered houses, and were seldom in many places in the same year." Elsewhere the same author states that the later outbreaks of plague in England resembled the Black Death in being "universal and in the homes of the peasantry" until 1407 or perhaps

[1] Nathan, *loc. cit.*

[2] See Haeser, *Geschichte der Medicin und der epidemischen Krankheiten*, pp. 142 and 170.

[3] *History of Epidemics in Britain* (Cambridge, 1891).

1439. "From that time onwards town and country are contrasted in the matter of plague; it became usual to flee to the country so as to escape the pestilential air in town in the summer heats[1]." Creighton also quotes many instances in which the mortality was so great in the country that no heirs existed to inherit estates, or peasants to till the ground. No evidence of such depopulation exists for London or other towns.

Thus all the known plagues of Western India resemble the Black Death and the epidemics to which it gave rise, in showing a high degree of intensity at one time over a large area, and in the relatively high rate of mortality that they produced in villages as compared with towns. The two groups of outbreaks also resemble one another in their power of spreading with facility from village to village. With regard to the extent of area attacked, we find, it must be admitted, a contrast. But in this respect the present Bombay plague may be regarded as intermediate between the Black Death and the Pali plague. Correlated with the high degree of virulence, there appears to be a certain similarity in the symptoms observed in these outbreaks. When some years ago a controversy arose as to whether Levantine plague was the same disease as the Black Death, the matter was settled by reference to the plagues of Gujerat and Pali, in which the pneumonic form of the disease had been observed, as happened during the Black Death, and as was not usually the case in plague of Levantine origin in modern times.

From these considerations it follows that some of the arguments that have been relied on above to support the idea that Western Indian plagues have a common origin in Garhwal, must fall to the ground unless it can be shown that the Black Death may also have been a result of the wanderings of Garhwali fakirs.

Probable Indian origin of Black Death.

No satisfactory suggestion has as yet been put forward as to the original source of the Black Death. It will therefore be of interest to discuss the possibility of its having come from Garhwal.

More than one contemporary historian relates that the Black Death was brought to Europe from the Crimea. But the exact circumstances of this event were unknown until the discovery in 1842 by Henschel of a most interesting manuscript in the Rhediger Library at Breslau[2].

[1] *loc. cit.* p. 233.
[2] Haeser, *loc. cit.* Vol. III. p. 157.

The author of this manuscript was Gabriel De Mussis, a notary of Piacenza, who was employed by the merchants trading in the Crimea. At the time in question these merchants were attacked by the Tartars, and besieged in the town of Tana on the river Don. They had to retire from this place to the town of Caffa on the sea-coast. Here they were besieged for nearly three years. Suddenly "the death" broke out in the Tartar host and thousands were daily destroyed "as if arrows from heaven were striking at them and breaking down their pride." The Tartars, hoping to communicate the infection, threw the bodies of the dead into the town by means of their catapults. The disease soon broke out among the besieged and they had to evacuate the town in their ships.

Constantinople was the first port reached by the fugitives, and their arrival was shortly after followed by the appearance of the disease. The Emperor, John Cantacuzenes[1], wrote an account of the pestilence which is still extant, and in which he states that it came from among the Tartars in the Crimea.

After leaving Constantinople the merchants touched at Messina in Sicily. The consequences of the arrival of the infected ships are thus described by a Franciscan friar, Michael Platiensis:—"A most deadly pestilence sprang up over the entire island. It happened that in the month of October, in the year of our Lord 1347, about the beginning of the month, twelve Genoese ships, flying from the divine vengeance which our Lord for their sins had put upon them, put into the port of Messina, bringing with them such a sickness clinging to their very bones that did anyone speak with them he was directly struck with a mortal sickness from which there was no escape."

Three of the plague-stricken vessels, on one of which was De Mussis, put into Genoa in January. A few days after their arrival the disease appeared in Genoa, although no infection was known at the time to have been present on the ships. It is related that hardly a seventh part of the population of the town was left alive.

The plague was brought to Venice by another of the infected vessels. From this place and from Genoa the disease rapidly spread over the whole of Italy.

As soon as the Genoese authorities recognised that the ships were the source of infection they compelled them to leave the port. One of them is known to have gone to Marseilles, and to have introduced the

[1] Haeser, *loc. cit.* p. 161.

plague into France. In one month the disease is stated to have carried off 57,000 inhabitants of Marseilles and its neighbourhood.

Black Death not derived from China.

In view of the opinion frequently held that the Black Death came from China, it is important to notice that the Chinese annals contain no mention of any such disease in the years preceding the appearance of the Black Death in Europe. On the other hand great plagues are mentioned in the Chinese annals in the years 1352 to 1363[1].

According to De Mussis it commenced, not in the besieged town of Caffa, as one would expect to have been the case had the germ been imported in bales of merchandise from China, but among the surrounding Tartars. In spite of the statements of De Mussis it is probable that ships from other ports in the Black Sea than Caffa also had to do with importing the infection into Europe. The disease seems to have reached both Sicily and Italy in 1346, but was not known to have been present in Constantinople till the spring of 1347.

The plague, according to Ibn Batuta, reached Jerusalem in the spring of 1348, and Damascus only towards the end of July of the same year. Aleppo and Gaza were attacked in June. Hence this evidence indicates that the plague was present among the Tartars in the Crimea before it was present in Syria. That is to say, it had not followed the ordinary mode of origin of Levantine plague.

The Arab historian Aboel Mahasin[2] states that the plague began in Tartary and travelled thence to the Tartars in the Crimea, and then on to Constantinople and Europe, and in another direction to Asia Minor, Syria, and Egypt.

Indian outbreak antecedent to Black Death.

Having thus traced the Black Death back to Tartary, it is necessary to see whether the records of Indian history contain any mention of a pestilence shortly before that time (1346). As we have seen, plagues in Western India have occurred in years of Nassik festivals. Analogy would therefore lead one to suspect that the Black Death, if of Indian origin, should have commenced shortly after one of these twelve-yearly festivals. Counting backwards by twelve-yearly intervals we find

[1] Creighton, *loc. cit.*

[2] Quoted by Des Guignes in *Histoire des Huns.*

that a pilgrimage must have occurred at Nassik in August of 1344, and in view of the great antiquity of Indian religious customs[1], we may safely surmise that in the spring of that year numbers of fakirs emerged from Garhwal, and travelled by various routes through the plains of India to the sacred shrine. But on referring to Indian histories no explicit mention of plague in that year could be found. Elphinstone[2], however, states that a rebellion broke out in Ma'bar in 1341, and that an army sent by the Emperor of Delhi to suppress it was so wasted by the ravages of a pestilence that it was forced to return[3]. This statement seems to imply that the pestilence appeared soon after the rebellion, namely in 1341, and hence could not be connected with the movements of the fakirs in 1344. But a further examination has shown me that the contrary is the case. In a contemporary history, known as the *Tarikh-i Firoz Shahi*[4], it is stated that on receipt of news of the rebellion an army was despatched to Ma'bar. Afterwards the Emperor returned to Delhi for reinforcements. When he again left Delhi, probably in the autumn of the year, a famine was commencing. When the Emperor was still three months' march distant from Ma'bar, according to the traveller Ibn Batuta, pestilence broke out in the army, and the greater part of it perished. The Emperor himself and many nobles were attacked. After halting at Deogiri (a few marches east of Nassik) while suffering from the disease the Emperor returned to Delhi. He travelled through Malwa, by what appears to have been the ordinary route to Ma'bar, and reached Delhi still weak from his illness (*Tarikh-i Firoz Shahi*)[5].

[1] Alberuni, a Persian writer, was born in A.D. 973, and lived at the court of Mahmud of Ghazni. In his description of India he refers to "the Tree of Prayaga...the place where the waters of the Jaun join the Ganges, where the Hindus torment themselves with various kinds of tortures." Prayaga is the place now known as Allahabad. With little doubt his statement refers to the tricks of fakirs at the pilgrim festival that is still held there. See Alberuni's *India* by Dr E. C. Sachau, London, 1888, Vol. I. p. 200.

[2] *History of India*, Fifth Edition, 1866, p. 406.

[3] The word "waba" used to describe this outbreak is translated as "cholera" in Elliot's translation of Ziaud Din Barni. The word is more usually translated as pestilence and is commonly employed for plague. The known comparatively recent origin of the chief cholera deity in the United Provinces is one reason, among others, for doubting whether at the time we are discussing cholera was so prevalent a disease as it has since become. From Ziaud Din Barni's and also from Ibn Batuta's accounts it appears that the Emperor, when attacked, remained ill from the pestilence for some months, a fact that agrees better with the view that the malady was plague, and not cholera.

[4] By Ziaud Din Barni. See a translation in Elliot's *History of India by its own Historians*, Vol. III. p. 243.

[5] *loc. cit.* p. 244.

When he arrived the famine was at its height. According to the contemporary historian, Ziaud Din Barni, "Not a thousandth part of the population remained. He found the country desolate, a deadly famine raging, and all cultivation abandoned...and man was devouring man." According to Elphinstone, the date of this famine was 1344, and as shown by the above extracts it must have occurred in the same year as the pestilence in the army. Further the year 1344 is given as the date at which the rebels in Ma'bar succeeded in throwing off the authority of the Emperor, and establishing an independent kingdom. This also harmonises with the date given for the destruction of the Emperor's army. Thus this pestilence might have been carried by the commerce in horses and merchandise that then existed to Bokhara and Samarkhand[1] in time to have been the source of the Black Death among the Tartars in 1346[2]. An analogy for so distant a spread of Indian plague appears to exist in the plague of 1611, which commenced in the Punjab and spread to Kashmere and Kandahar.

Thus the resemblance of the Black Death to plagues in Western India does not invalidate the arguments in favour of a common origin of the latter outbreaks based on their epidemiological similarities.

Since writing the above paragraphs I have come across a definite statement by an Arabian author to the effect that the Black Death came from India. Ibn Wady, who is known as a historian by his continuation of the annals of Abulfeda, says that the disease first arose in "the Land of Darkness." Thence it spread to China and India. From India it spread to the land of the Usbeks and Transoxiana, reached Persia, devastated Central Asia, the Crimea, the Byzantine Kingdom, then Cyprus and the Islands. Then the disease reached

[1] Referring to Akbar, Abul Fazl makes the following statements: "His Majesty being very fond of horses, merchants bring them from the two Iraks, Room, Turkestan, Badakshan, Shirvan, Khergez, Tibbet, and Cashmere." (*Ain-I-Akbara*, Gladwin's Translation, Vol. I. p. 130.)

[2] According to Ziaud Din Barni this pestilence broke out at Arangal (spelt by Elphinstone Warangal). According to Ibn Batuta it commenced at Badrakote. In either case it is probable that the infection was brought to the army by reinforcements that had travelled by the ordinary route through Malwa, and crossed the Nerbudda and Tapti rivers, that is to say, who, for some distance, had travelled along the same route as the fakirs on their way to Nassik. The fact that the Emperor halted at Deogiri (on the Godavary below Nassik), and that he was still weak from his illness when he arrived at Delhi three months later, strongly suggests that he was infected not far from the former locality. For the route followed by an army from Delhi that originally conquered Ma'bar in A.D. 1310, see the *Tarikh-i Alai* by Amir Khusru, translated in Elliot's *History*, Vol. III. pp. 86 and 87.

Egypt, desolating Cairo and Alexandria. It extended to Upper Egypt, spread westwards along the African coast to Barka. In the other direction it went through Gaza and Askalon to Syria. It attacked Acca and Jerusalem. At the same time it passed along the coast to Saida and Beyrut. Thence it spread to Damascus. After infecting many other places, which the author mentions, it reached Aleppo, where Ibn Wady himself witnessed its ravages[1].

Means of spread of plague.

It is now necessary to consider the means of spread of the plague infection. Is it by direct contagion from patient to patient? Or from the dejecta of infected rats to human beings? Or does an infected insect play a part in the transmission of the disease?

That the process is not a simple one is indicated by the following considerations.

Simond's latent period in the locality.

When the infectious material has been brought into a village it frequently does not manifest its activity until after a period of weeks or even months, as was first pointed out by Simond for plague in Western India[2]. Both in the Bombay Presidency and in Garhwal the typical mode of development of an outbreak of known history is as follows. The person bringing the infection is, usually, himself attacked, and also a varying number of those in contact with him, within a few days of his arrival, and within the probable incubation period of the disease. The virus then remains quiescent for a long period, generally for about twenty days, but sometimes as little as ten days, and sometimes for a longer period extending to three or more months. The first sign of its renewed activity may be the death of rats, human beings only falling victims after these rodents have been killed off. In other cases rats and men are attacked simultaneously, or lastly only men may be attacked. In the case of Garhwal the phenomena observed by Dr Planck[3] are specially curious. According to this author, outbreaks due to a proved (and recent) importation of the malady are never accompanied by a mortality among rats. On the other hand, "spon-

[1] See Kremer, "Ueber die grossen Seuchen des Orients nach arabischen Quellen," *Sitzungsberichte der Philos. Histor. Cl. d. Kaiserl. Akad. d. Wiss.* Bd. XCVI. p. 69, Vienna, 1880. (I am indebted for this reference to Mr A. W. Thomas.)

[2] "La propagation de la Peste," *Annales de l'Institut Pasteur*, October, 1898, p. 625.

[3] *loc. cit.*

taneous" outbreaks (which may be due to an importation of the virus a long time previously) are often preceded or accompanied by such mortality.

This undeniable fact of the frequent occurrence of a long period of incubation in the locality independently of the incubation period in the human body, indicates that, as a rule, plague spreads, not by simple contagion from patient to patient, but by some deep-seated and perhaps complicated method.

Anomalous outbreaks.

The complexity of the phenomenon is indicated by curious cases in which certain classes of the population or species of animal escape. For instance in Bombay, as I believe is usually the case in plague in India, rats were attacked, while mice escaped. But at Bandora near Bombay, both mice and rats were infected. Plague in Jeddah (probably derived from the Assyr endemic area) in 1897 was accompanied by a mortality of both rats and mice. In other cases, as will be shortly shown, there can be little room for doubt that rats and mice have completely escaped the epidemic[1]. Certain outbreaks appear to have shown a special severity among children.

[1] The outbreak of plague in Kankhal exhibits strikingly the phenomenon of the disease attacking different species of animals at different periods. The infection appears to have been introduced on the 14th May, 1897, by a priest who had been present at the disinfection of a house in Hurdwar on the 7th of May. He died on the 16th May in Kankhal. No further plague was reported till about the 20th June, when an outbreak among rats, bacteriologically diagnosed, occurred in the locality where the first human case had died. The succeeding outbreak among human beings is suspected to have commenced, in the same locality, on the 3rd or 4th August, 1897, though the first case definitely diagnosed occurred on the 6th September. The outbreak among human beings comprised 61 cases, and lasted till the 6th January, 1898. An outbreak of plague among monkeys, bacteriologically diagnosed, began about the middle of October, 1897, and lasted for about a fortnight. Twenty-five dead bodies of monkeys were found, but it is supposed that a larger number were attacked, as when ill these animals are reputed to go into the jungle to die alone. As a precaution about 650 monkeys were caught and kept in cages until the epidemic was at an end. Other monkeys emigrated from Kankhal, and destroyed crops near the village of Jaggitpur, about a mile and a half distant from the town. It was suggested that these monkeys may have been the cause of the epidemic of human plague in that village, that commenced, so far as is known, on the 29th December, 1897, and amounted to 23 cases of the disease. (See *Evidence before the Indian Plague Commission*, of Mr Winter, Vol. II. p. 42, and of Mr Kendall, Vol. II. p. 58.)

"Pestis Puerorum."

The pestilence of 1359 and the following years seems to have marched through a great part of Europe, like the Pied Piper of Hamelin, accompanied, if not as in his case by a disappearance of the rats, at all events by a destruction of children, and is described by more than one contemporary historian as the "pestis puerorum[1]." In Poland it is stated to have attacked chiefly the upper classes and children (1360). In France (1361) it is stated by Guy de Chauliac to have destroyed innumerable children and many members of the upper classes, including five cardinals and a hundred bishops. Two English historians state that the plague of 1361 was known as the "pestis puerorum," but bishops and nobles are recorded to have been among its victims. In a few other exceptional instances the bubonic plague is stated to have shown a tendency to attack children more than adults. For instance the Plague of Justinian appeared in Constantinople in A.D. 542. In A.D. 556 many towns in the Byzantine Empire were again attacked and the disease is stated to have been especially severe among the young. A restricted epidemic in November and December, 1898, and January, 1899, in a suburb of Kurrachi (*i.e.* during the interval between the second and third epidemics in that town) affected 13 children out of a total of 22 attacks. In London in 1382, a disease, probably the bubonic plague, was especially severe among children.

Both in the Black Death and in Indian plague it has been noticed that persons of filthy habits or occupation have escaped the disease. At the commencement of the Bombay outbreak the disease was almost confined to very cleanly castes, whose members as a religious duty never touch food without extensive ablutions. The scavengers remained almost completely exempt from the disease, though their duties must have constantly brought them into contact with the dejecta of plague patients. A similar fact was observed by Dionysius Colle during the Black Death in Italy. He states that "Coriarii qui latrinas exportant,

[1] Since writing this sentence I have had the curiosity to look up the authorities for the story of the Pied Piper. According to Verstegan, the first English writer to describe the incident, it occurred in the year 1376. But according to the brothers Grimm (*Deutsche Sagen*, 1816, Vol. I. pp. 330—333) it happened in the year 1284. According to the story, the Pied Piper appears to have been an indigent person who had witnessed a disappearance of rats in the dominions of the Cham of Cathay whence he had come. After his arrival at the town of Hameln in Brunswick, there was a disappearance first of the rats, and then of the children, the number of the latter being 130. In the following year, according to Schnurrer (*Chronick der Seuchen*) there was so great a mortality in Italy, Lombardy, and Apulia, that many bishops and prelates remained unburied. In 1284 there had been a severe plague outbreak in Egypt. For other authorities see Furnival, *Bibliography of Robert Browning*, pp. 113 and 158. From the facts that tradition associated the event with a particular street in Hameln, and that the archives of the town for some centuries were dated from the time of the disappearance of the children, it appears to be probable that the legend was based on an importation of plague rather than on an ordinary folk-lore tale.

hi etiam, qui xenodochiis inserviunt et locis foetore gravi molestis, omnes fere a peste immunes conspiciebantur: venenum enim venenis debellatur, arcetur, et expellitur[1]."

Rats not a necessary cause or agent in the spread of plague.

Evidence obtained during the Bombay outbreak, as also the evidence from Garhwal, leaves little room for doubt that rats are not a necessary factor in the spread of the plague. In Garhwal out of forty outbreaks investigated by Planck a rat mortality was only observed in eight. This is strong evidence, as the inhabitants knew well the meaning of the sign, and had, in nearly every case, no motive for concealment. In the Bombay Presidency cases occurred in which the same class of observers at one time noticed, and at another time failed to discover the phenomenon. For instance in Hubli, at the time of the commencement of the disease, outside the town near the railway station, numerous dead rats were found. When the disease spread through the town, despite careful search, dead rats were never observed. The Black Death appears to present an analogous phenomenon. When it reached Constantinople it was accompanied by a mortality among rats, but no such phenomenon was recorded during its spread through the rest of Europe[2]. A striking case is that of Kolobouvka in Astrachan, one of the few cases recorded of virulent plague being dealt with successfully. Here 3,000 rodents of different species were examined bacteriologically without the plague microbe being detected in a single case. The officials on the spot ascribed their success in dealing with the outbreak to the fact that it did not extend to rats. The question of the probable relation of rats to the spread of plague will be further discussed in a later paragraph.

[1] Haeser, *loc. cit.* p. 170.

[2] See Abel, "Was wussten unsere Vorfahren von der Empfänglichkeit der Ratten und Mäuse für die Beulenpest des Menschen" (*Zeitschr. f. Hygiene und Infectionskrankheiten*, Vol. XXXVI. p. 89). Abel shows that supposed references to a connection between rats and plague in European medieval authors are for the most part based on a quotation from Avicenna. There is no adequate reason according to Abel for believing that any noticeable rat mortality ever accompanied plague in the Middle Ages in Europe. Eastern authors not infrequently refer to rats staggering about as if drunk in times of plague. In Chaucer's *Canterbury Tales*, in the Knightes Tale (circa A.D. 1380); I noticed the phrase "as dronke as a mous." It would be interesting to know the origin of the expression.

"Contagionist" and "localist" theories of plague.

In a discussion on the probable mode of the spread of the plague virus it will be advisable to consider the bearing of modern discoveries on the controversy between "contagionists" and "localists" as to the nature of the disease.

The "localists" believed that plague was due to a miasma bred on the spot where the disease was manifested. In support of their views they relied on certain facts that tended to show that the disease was not contagious.

The "contagionists," on the other hand, believed that the disease was due to a contagion imported, by means of a plague patient, from some other place where the disease existed. They relied on certain facts that tended to show that the disease could be caught from a patient.

The researches of Bitter[1] first indicated how modern bacteriological knowledge could explain away certain facts relied on by the localists, or bring them into harmony with the contagionist view of the disease.

Bitter showed that in certain cases the microbe remained confined to the bubo. Such patients were therefore not likely to be a means of transmission of the disease. In other cases, on the other hand, the microbe travelled from the bubo to the blood of the patient, and thence to the excreta, and hence such excreta *à priori* appear liable to transmit the infection.

In 1835 Clot Bey, a French doctor, inoculated himself with pus from a plague bubo[2]. He remained unaffected. This, as shown by Bitter, does not disprove the contagionist doctrine, for it generally happens that at the period of suppuration plague bacilli are no longer present in the pus. In 1802, White, a surgeon serving with the English army in Egypt, inoculated himself from a plague bubo, and died of plague on the seventh or eighth day afterwards. In this case plague bacilli must have been still present if the inoculation was the cause of the illness.

Although the discovery of plague bacilli in the dejecta of patients appears to settle the question of their contagiousness in a positive sense, it may be pointed out that from the practical standpoint, the contagionist view is by no means accepted as regards an established epidemic. In practice it has been found in India that the infected locality is a far greater source of danger than the plague patient.

[1] *Report of the Commission sent by the Egyptian Government to Bombay to study Plague* (Cairo, 1897), p. 64.

[2] Proust, *La défense de l'Europe contre la Peste*, p. 161.

Hence the policy of evacuation, which measure may be regarded as harmonising with the "localist" theory of plague. On the other hand, as regards places not yet infected, the policy adopted is "contagionist." The thing to be feared is the actual importation of the infection by a plague patient or infected person. No one now-a-days would believe that the plague virus originates *de novo* as a miasma bred from an accumulation of filth.

Plague a "miasmatico-contagious" disease.

The question arises whether from the theoretical standpoint also plague should not be regarded from this combined contagionist and localist point of view, whether, in other words, it should not be regarded as belonging to the group of miasmatico-contagious diseases, such as cholera or enteric. In the case of cholera we know that the disease is carried from place to place by human intercourse, if not by infected persons. The microbe is usually present in the dejecta of the patient, but during an outbreak the dejecta are not the usual source of infection. An imported cholera patient is not likely to be the origin of a severe outbreak unless the microbe contained in his dejecta succeeds in reaching the public water-supply. Should this happen isolation of the patients as fast as they occur will not bring the outbreak to an end. It is necessary to evacuate the locality, or at any rate to change or purify the water-supply. Similarly, with plague, the question arises, whether during the outbreak the dejecta of the patient are the chief source of infection, or whether, on the other hand, the microbe in the dejecta is not usually dangerous unless and until it reaches some special "nidus."

Dejecta of human patients not an important source of infection.

It should be recognised that the belief that the dejecta of patients are actually the source of infection, is a deduction drawn from the fact that they frequently contain plague bacilli. It is not an induction based on definite *à posteriori* proofs, except as regards a few cases of apparent infection from sputa in the pneumonic form of the disease, and a few curiously limited outbreaks from this source. It is difficult to accept the view put forward by Bitter that pneumonic cases are of greatest importance in spreading the disease, in view of the fact that the pneumonic form of the disease was unknown in certain outbreaks in the Levant that showed great power of spreading, while it formed

a large proportion of the cases in the Pali and Gujerat plagues that spread over only a very restricted area. In the recent plague in Alexandria the complete absence of known infection from pneumonic cases attracted the attention of observers on the spot.

The view that the plague infection in a house is due to the microbes contained in the dejecta of a patient does not harmonise with the fact that, in certain cases, the infection of others does not take place until a period of from twenty days to four months has elapsed from the time of arrival of the first patient. It is difficult to reconcile this latter fact with the view that infection is simply due to the passage of the microbe from the dejecta of the patient to another person through a crack in the skin. Nor does this view harmonise with the fact that there was a great exodus of actual sufferers from plague from Bombay in December, 1896, to every portion of the Bombay Presidency, while the disease did not commence to spread widely through the different districts until September, 1898, at which time, owing to the stringency of the regulations, it had long been impossible for plague patients to travel, and "suspected persons" were liable in most cases to a certain amount of detention.

It is admitted on all hands that only a portion of the actual exported plague cases, during this earlier period, were discovered, owing to the hostility of the population to plague measures. The following facts may be brought forward as additional proofs of the wide spread of plague cases without a concomitant spread of the plague infection at this time in the Bombay Presidency[1].

Before the discovery of any indigenous cases:

In the Broach	District	23	cases of imported plague were discovered between	Oct. 1896 and March 1898.
,, Kaira	,,	105	,, ,, ,, ,,	Dec. 1896 and Sept. 1898.
,, Ahmednagar	,,	72	,, ,, ,, ,,	Dec. 1896 and Sept. 1897.
,, Khandesh	,,	37	,, ,, ,, ,,	Dec. 1896 and June 1897.
,, Nassik	,,	47	,, ,, ,, ,,	Dec. 1896 and May 1897.
,, Kolaba	,,	41	,, ,, ,, ,,	Oct. 1896 and Jan. 1897.
,, Hyderabad (Sind)	,,	33	,, ,, ,, ,,	Jan. 1897 and Feb. 1897.

The greater number of these imported cases of plague occurred in December 1896. In the case of Nassik Town, it is recorded that "no less than 26 cases of a virulent type of the disease (these cases showed a mortality of 92·73 per cent.), nearly all in the same quarter of the town (Kajipura and its vicinity), in which the conditions seemed to be particularly favourable for its becoming indigenous, occurred, before

[1] Condon, *The Bombay Plague, being a history of the progress of plague in the Bombay Presidency from September* 1896 *to June* 1899. This important official report contains a vast amount of information. I have made extensive use of it in this and the following paragraphs.

one of the local residents contracted the disease." It is impossible to say how many of the discovered cases in the above list were isolated, or in how many cases their dejecta were disinfected. The point on which stress may be laid is, that from the conditions of the case, the panic among the inhabitants of Bombay, the sudden extension of the disease in that town, &c., it is probable that at least as many exported plague cases were overlooked as were discovered. In these undiscovered cases no attempt at isolation or disinfection was made. The dejecta would fall on the mud floors of native houses: the relatives of the patient would even receive his sputa in their hands, wiping it off on to their clothes or on to the walls or floor of the room, as was most convenient. Nevertheless in the great majority of these instances the relatives remained in these "infected" houses exempt from the disease, in strong contrast to what would have been their fate had they lived in "disinfected" houses in a village in which plague and plague operations were in progress.

Thus at one period of the Bombay outbreak there was exportation, and on a very large scale, of infected persons, without resulting local infection. On the other hand, at later periods of the Bombay outbreak, not only was the disease often exported by persons themselves attacked, but also by persons who either escaped the disease or who were only attacked at a later period.

Persons carrying the infection dangerous in villages, comparatively innocuous in camps.

Experience gained both in India proper and in Garhwal shows that a person carrying the infection is a far greater source of danger if he goes to a village than if he goes to a temporary habitation, such as a sanitary camp. The difference in results does not appear to be altogether due to a stricter supervision in the camps, though it is difficult to estimate exactly how far this factor may be operative. In the case of villages, despite immediate removal of the person bringing the infection, and disinfection of the surroundings (as at Kankhal), the event may be followed, after an interval of weeks and months, by an outbreak of the disease. In the case of camps the importation of the infection is rarely followed by more than one or two cases of the disease, and no prolonged incubation period in the locality has been observed. This appears to be the case even if the inhabitants have entered the camps without any previous disinfection of their clothes or goods, or even where from the necessities of the case the camps have been over-crowded or otherwise in insanitary conditions, and though the time of residence may extend to several months. After persons have resided for ten days in sanitary camps it appears to be safe to permit them, at

all events after disinfection of clothing, to go to other villages. Thus, it is probable that cases of prolonged incubation periods in the locality are not due simply to the microbe having under natural conditions a greater power of resisting desiccation, &c., than it appears to have in the laboratory; on the contrary they appear due to the fact that some condition is present in villages that is favourable to this slow development of the plague infection, which condition is not usually present in temporary camps, or at least not present to so great a degree as in villages. That the condition in villages favourable to the development of plague is the presence of rats is an obvious suggestion. As an apparent proof of its correctness the history of the outbreak in the village of Mahlgahla in the Punjab in the year 1898 may be cited.

Spread of infection independently of human aid.

The infection was introduced by a woman who was attacked by the disease, and who on the day following her attack, with her whole family, was removed from the village to an isolated camp. Twenty-one days later seven of the inhabitants of the village were attacked. All the inhabitants were then put into camp, and the disease apparently came to an end seven days after this had been done. The persons up till then attacked were members of the Chamar caste, and lived in a separate quarter of the village. Four days after the disease had apparently stopped, disinfection of the evacuated quarters of the village in which no human cases of plague had occurred, was commenced. Numbers of dead rats were found in each of these quarters. Consequently there was another outbreak of plague, amounting to 76 attacks, chiefly among persons engaged in disinfection, and the disease only came to an end when disinfection was stopped. Persons disinfecting the Chamar quarters were not attacked, but infection was rife among those who entered other parts of the village in which no human cases, but merely a rat mortality, had occurred. Consequently in this case the infection spread through the village independently of human aid, and as it would appear, at first sight, owing to the agency of rats.

Plague in Kankhal and Jawalapur.

But that this conclusion is not necessarily correct is indicated by the history of plague in the adjoining towns of Kankhal and Jawalapur in the United Provinces[1]. In these cases there can be no doubt that the disease spread through each of the towns independently of human influence. In these towns, as soon as a case occurred, not only the relatives of the patient, but also the inhabitants of the whole of the

[1] See *Evidence before the Indian Plague Commission*, Vol. II. p. 50.

surrounding quarters of the town, were turned out into a sanitary camp. On several occasions, after a block comprising some acres in extent had been evacuated, the next case occurred after an interval of ten to twenty days in a house on the margin of the evacuated area. To the officials on the spot the plague appeared to spread centrifugally from the original infected centre as if carried by some creeping insect. In neither case was any direct evidence obtained that rats played a part in the spread of the disease. In the case of Kankhal, a rat mortality occurred between one and two months before the commencement of the disease among human beings, and during the subsequent disinfection of the town neither living nor dead rats were found. In Jawalapur, on the other hand, no rat mortality was observed, either before or at the commencement of the outbreak among men, and during the disinfection of the town living rats were observed. The outbreak began in January among men, and no mortality among animals was observed till March, when plague was bacteriologically diagnosed in three rats and a few monkeys. The total number of human plague cases in Jawalapur was 116. Of these no less than 29 were of persons or of relatives of persons employed in disinfection, and consequently may be regarded as further proofs of the infection of the locality. Deducting these 29 cases, there remains 87 of the ordinary inhabitants of the town. Out of these 87, no less than 13 were grain dealers, or relatives of grain dealers. Of these 13 cases, 11 had occurred before the end of February, and the remaining two early in March. That is to say they all occurred at the commencement of the outbreak, and formed more than a third of the cases during this period. During the later and longer part of the epidemic the grain dealers remained exempt. What special liability can grain dealers have for plague except that due to the fact that their shops and houses are infested by rats?

Liability of grain dealers to contract infection.

The liability of grain dealers to plague in India has often been observed and quoted as a proof that the spread of the disease is due to rats. But if the validity of this argument is admitted, surely the converse must be true, namely, that an immunity of grain dealers to plague should be regarded as a proof that the spread of the disease is not due to rats. That is to say the facts recorded for the Jawalapur outbreak indicate that at its commencement the spread of the disease

was due to rats, while at a later period its spread was due to some other agency.

Precisely similar facts were noticed during the outbreak in the town of Bombay, though owing to concealment of cases the statistics are less reliable than in the case of Jawalapur. Existing figures however show that at the commencement of the outbreak the grain dealers furnished more cases than the members of all other trades dealing with other provisions than grain taken together. Later, in November and December of 1896, they showed no such preponderating susceptibility.

Migration of rats.

It may be replied that the phenomena observed are simply due to an emigration of infected rats. At the commencement of the outbreak they lived in grain dealers' houses. Later they migrated carrying the infection to other houses. In the case of Bombay there is no doubt that such migration occurred. Further, in nearly every district of the town the plague assumed epidemic form at an interval varying from two to six weeks after the first observed death of rats. But it is not clear why the migrating rats did not carry the infection especially to grain dealers' shops in other quarters of the town. Neither does the suggested relation apply to numerous other outbreaks in the Bombay Presidency where no special liability of grain dealers was observed.

No quantitative relation between rat and human mortality.

Yet another line of argument may be brought to bear on the subject. If two phenomena are causally related, we may expect that a variation in one should be followed by a variation in the other. To apply this rule to the matter under discussion, if the disease among rats was the cause of the disease among men that followed it, in so many instances at least, in Bombay, one would expect that where more rats died more men would be attacked, and *vice versâ*. The case of the grain dealers in Mandvie (the first district of the town attacked) may be cited as a strong argument of this kind. But this was only the case at the commencement of the outbreak. During October in Mandvie rats died in other places than in grain dealers' shops. Children amused themselves by throwing the dead rats out of the windows, and I recollect during this month seeing heaps of dead rats that were about to be destroyed in the streets. No such sight was to be seen

in any other district of the city either then or later on. Dead rats used to be observed in various districts as the disease progressed, but never in such numbers as were seen in Mandvie. But these other districts often suffered far more severely than Mandvie from the plague. The Municipal Commissioner in his report states that vast numbers of rats appeared at the end of December in the northern districts in places where they had never been seen before, and regards their appearance as a cause of the severe outbreaks in the suburban villages. But no proof is given that rats died in any great numbers in these places. I visited several infected villages in these localities during January, and my enquiries elicited no definite proof that dead rats had been observed, though some are stated by Weir to have died in Mahim. The migrating rats seem to have disappeared. This, the Municipal Commissioner states, was due to their having travelled still further in a northerly direction across the bridges and causeways from Bombay Island to the mainland, where he believes them to have been the cause of further village outbreaks. In Worli village (north of Bombay) after the place had been evacuated I saw musk rats, which appear to have remained everywhere unaffected by the pestilence.

Agency of rats of different degrees of influence at different periods of outbreak.

In view of the facts here summarised we are led to the conclusion that if rats played a part in spreading the infection, they must have done so to very different degrees at different periods of the epidemic. A precisely analogous conclusion has been arrived at by Gotschlich from a study of the Alexandria outbreak[1].

Interval between mortality of rats and mortality of men.

That the plague is not conveyed directly (to an appreciable extent) from the dejecta of infected rats to men is proved by the fact already noted that the disease only assumed epidemic form among human beings two to six weeks after the first observed mortality among rats in most of the different districts of the town of Bombay. It is further proved by the fact that the disease continued in most districts among human beings long after the rat mortality had come to an end.

[1] See *Zeitschr. f. Hygiene*, Bd. xxxvi. p. 202.

For instance, the Municipal Commissioner's Report states, "By the middle of March (1897) not a rat was to be seen or heard on Malabar Hill; and yet in ordinary times they infest the whole locality and are constantly appearing or making their presence known. They have never returned, or only such a few as to be unnoticeable (up to 2nd October, 1897). Since last March, on the Ridge, where they were very plentiful, I have never seen a rat, and only a few of the musk tribe remain." But plague among human beings in this locality continued till the middle of April, 1897. The Municipal Commissioner states also that: "By the commencement of December nearly all the rats had disappeared from Mandvie and adjacent quarters of the city, while they were noticed in Kamathipura, Tardeo, and Byculla, in great numbers, many of them being found dead. The bubonic plague followed in their track with unerring regularity." But the plague continued in Mandvie and the neighbouring districts till the month of May. It came practically to an end at the same time in the districts of Tardeo, Byculla, and Kamathipura, though these localities had been so much later infected.

Attachment of infection to locality.

A class of facts tending to prove that plague is not usually due to simple direct infection from the dejecta of human patients is the attachment of the infection to the locality. Both in Jawalapur and Kankhal the cases of plague that occurred among recent arrivals in the segregation camps, did not, except in isolated cases, form foci for the further spread of the disease. On the other hand, during this time, the infection was active and spreading in the locality from which these patients had been brought, as evidenced by the following facts:

"The second area evacuated in Kankhal contained the house of one of the leading men in the town. The house was well-built, and ventilated, and kept scrupulously clean. For various reasons we made an exception in favour of this man, and allowed him and his family to remain in their house. Two members of the family were attacked, although all the surrounding houses had been evacuated. A man was attacked by plague in a house in which a woman was about to be confined. The family of nine persons were segregated in their own house, the surrounding houses being evacuated; five members of the family were attacked. In another block that was evacuated there was a house in which a woman owing to recent confinement could not be moved. She with two attendants were left in the house. Her sister was attacked within a week of the evacuation. An old man and his sister were left in an evacuated area to look after a temple. The sister was attacked with plague two months after the surrounding houses had been evacuated, and after the whole town had been evacuated. After the outbreak had to all appearance ceased, four cases occurred in a family living in a collection of huts separated from the town, which for this reason had not been evacuated. Two chonkidars (watchmen) in evacuated areas were attacked. Large numbers of men employed in disinfecting evacuated houses were attacked, these attacks occurring for some time after the town or village had been evacuated.... There was no recrudescence in

Kankhal, but there were two cases imported from Jawalapur. We got a report when these cases occurred, and made enquiries, and found that the people concerned owned houses in Jawalapur, that within three or four days before they were attacked those houses in Jawalapur had been disinfected, and that, in accordance with the usual custom, a member of the family had been to Jawalapur to be present during the disinfection. He was attacked within three or four days of his return. Those were evacuated houses which were disinfected, and not houses in which cases of plague had occurred. Immediately steps were taken to segregate the people. The people of Kankhal themselves were very anxious that there should be no case of plague, and they gave us information." (Mr Winter's evidence before the Indian Plague Commission, Vol. II., p. 50.) This evidence obtained from Jawalapur and Kankhal is especially valuable, because of the completeness of the organisation that was employed for detecting cases of the disease, and for dealing with the outbreak. Similar evidence as to the infection of the locality in Garhwal will be given in a later paragraph.

Spread of plague not due to infected dejecta of men or rats.

Thus, so far from the patient's dejecta being the main source of infection, known facts indicate that only in a small proportion of instances does the microbe in the dejecta pass into the condition in which it produces infection of human beings in Indian plague. Facts are even compatible with the supposition that this practically never occurs apart from certain pneumonic cases. The problem of the means of the spread of plague here indicated is by no means solved by a reference to rats. Though in some cases there can be no doubt that they play a part in the spread of the disease, other cases that have been brought forward indicate that the plague can spread and remain attached to a locality apart from this agency.

Historical evidence teaches us that the most virulent outbreaks recorded have occurred among populations that habitually wear boots and shoes, rather than among populations that go barefooted. This fact militates against the idea that infection is due to the entry of the microbe through fissures in the skin of the feet. Still less probable is it that this is a usual mode of entry of the microbe for rats, which animals, as we may well believe, but rarely cut their feet by treading on stones or thorns, and are by no means so liable to wounds from other causes as they sometimes are to plague. Laboratory experiments show that the plague microbe loses its infectious power by repeated passages through rats by subcutaneous inoculation. Should these experiments be further substantiated, they would furnish good grounds for doubting whether contagion from rat to rat, or from rat's dejecta to rat, is the

usual means of spread of the disease among these rodents, and to a less extent from rats to men. The apparently spontaneous limitation of outbreaks of pneumonic plague caused by direct infection indicates that the plague microbe may undergo a similar diminution of its infective power by repeated passages through human beings.

Thus it is improbable that the true "nidus" of the plague bacillus is either dirt, or rats, or men; though either of these agents may be concerned in the exportation of the disease from one locality to another, or may be responsible for a few and isolated attacks of the disease, and must, in any event, be regarded as suspect from the practical standpoint.

Infection not due to transference of infected blood by biting insects.

The general immunity to infection of attendants in plague hospitals makes it improbable that bugs and mosquitoes cause human infection by biting while their proboscides are still fouled with the blood of septicaemic patients. It is difficult to see why the proboscis of the flea should be more liable to transmit infection in this way, whether we are dealing with fleas that normally bite human beings, or fleas liberated from infected rats.

Simond[1] has suggested that fleas deposit dejecta at the moment of biting, and that the microbe contained in such dejecta is afterwards accidentally rubbed into the bite, and so causes infection. But if fleas can be dangerous in this way, why should not other biting insects that are present in plague hospitals similarly infect the attendants? Further, it may be doubted whether this theory adequately explains the prolonged incubation period in the locality and persistence of the infection so often observed in outbreaks of plague. In further illustration of this point I will quote in detail a description from Planck[2] of an outbreak in a Garhwali village. The outbreak was due to an importation of the disease, and as already stated such outbreaks in Garhwal are never accompanied or preceded by a mortality among rats according to the careful observations of Planck. We cannot therefore, in this case, invoke a chain of cases among rats to explain the infectivity of the houses which lasted for at least five months.

The facts are as follows: In November, 1876, a boy named Keshrua went to Balt village to fetch wheat for seed, and slept in a house in which was a woman

[1] *Loc. cit.* [2] *Loc. cit.*

suffering from plague, believed then by the villagers to be typhus. He returned to his village Sirar, and fifteen days later his sister was attacked. She died after three days. While she was ill Keshrua was attacked and died. Five days after this death his little brother sickened and died. Ten days later his father was attacked. The man's brother and daughter were attacked within a few days. The village was a comparatively large one, thirty-six houses being shown in the plan in Dr Planck's report. On the occurrence of the above cases of plague the inhabitants fled away to live in temporary huts on the village lands. The survivors of the infected families consisted of Usup Singh's wife and three children. No person of the village would approach them, and they were found living in a grass hut near the infected houses when Dr Planck visited the place on the 28th January. The villagers regarded these persons as doomed though no case of plague had occurred for about two months.

The villagers wished Dr Planck to burn all of the three infected houses that had been inhabited by the two infected families. These were the last three in a terrace of nine houses. A third brother Gunga Ram had however just returned to the village, and he refused to allow his house to be burnt. The other two houses were burnt on the 28th January.

No attacks had occurred during December. Besides burning the houses Dr Planck burnt the clothes and blankets of the remaining women and three children, giving them new clothing instead. A better hut was also provided for them. But on the 30th January the woman, Usup Singh's wife, was attacked and died on the 31st. The new hut in which she died was thereupon burnt; also all her new clothes and blankets. The new clothes and blankets of the children, and also of an aunt who had been persuaded to live with them were also burnt. A new hut and fresh food and clothing were again provided.

On the 2nd February Gunga Ram's son was attacked, and died after two days' illness. Gunga Ram had promised not to allow anyone to enter his house, but despite his promise he had sent the boy there in the evenings to tie up the cattle in the lower story, which was used as a cattle pen.

On the 8th February the above-mentioned aunt, a girl, and also another child, an infant, were attacked. Owing to the cold they had been unable to bear the misery of life in a hut, and at night time had gone into the cattle pen of Gunga Ram's house, which was no longer occupied by Gunga Ram's cattle since his son had been attacked. The infant died on the 10th, and the aunt on the 13th. The bubo of the girl suppurated and she recovered.

On the 12th of February Gunga Ram was attacked. After his son had died he had ventured to the village to live in a house above the one which had been spared, and which latter he often visited. He died on the 15th.

On the 27th February a woman was attacked. She had been in the habit of tethering her cattle at night in house No. 4 of the infected terrace. She recovered.

On the same date another woman, a widow, was attacked, but recovered. She also had been in the habit of tieing up her cattle at night in the infected terrace at house No. 5.

On the 13th March a young woman was attacked. She had lived in a detached house with the first of the above two women. She died on the 15th.

On or about the 25th March a boy was attacked who lived with his father and

sister in an isolated hut. He had been in the habit of going every day to tether cattle and fetch food from a house just above the infected terrace.

On the 31st March the father of this boy was attacked. Dr Planck does not state whether or not this man went to the village to fetch food for the family when the boy was attacked.

From the end of March the inhabitants carefully avoided the village site and all remained in good health. At the end of May the village was thoroughly cleaned, and early in June it was inhabited without ill effects.

Suggestion that the plague bacillus causes a disease of some species of flea.

The only view of the matter that appears to me likely to lead to an explanation of the facts is that the true "nidus" of the plague infection is some species of flea in which the microbe causes a slowly developing infection that at length renders the insect capable of transmitting the disease, and in which insect the virus can retain or regain its virulence.

Interval between time of reception of infection by flea and development of its power of transmitting the disease.

If Simond's view were true, namely, that the flea merely retains the microbe in its intestine and passes it out with its dejecta, one would expect fleas to be most virulent immediately after, or soon after, ingesting the blood of infected rats. The theory now put forward is that the microbe developes in the flea, and only after a lapse of time is in a position to reach the proboscis in the act of biting. This theory obviously presupposes an interval between the time of reception of the virus by the flea and the development of its capacity to pass this virus on to other animals. As explained above such an interval is usually observed in outbreaks of plague.

Changes in the habits of fleas as the rat population dies off may explain cases in which rats appear to play different parts in the spread of the disease at different periods of the outbreak.

Differences in the habits of fleas in different localities may be the cause of abnormal outbreaks in which certain susceptible species of animals temporarily or permanently escape. The class of facts here referred to, and which have been described in earlier paragraphs, are impossible to explain on the theory that plague transmission is simply a chance passage of the microbe from infected dejecta to accidental cuts or scratches on the bodies of susceptible animals.

Evidence that seasonal variations in plague outbreaks may be connected with habits of fleas.

Changes in the habits of fleas at different periods of the year may be the cause of seasonal variations of plague so frequently observed. The following facts may be quoted in support of this suggestion. In the spring of 1901 I was studying fleas obtained from cats and dogs in Agra, and found no difficulty in obtaining as many of these insects as I required. But at the commencement of the hot weather I found that the fleas on the cats living in my laboratory had suddenly and completely vanished. Recognising that the matter was of interest and wishing to subject it to an adequate test I at once offered a half-day's wages to my servants for every flea they could catch. Though the servants lived in different parts of the town and in surrounding villages not a single flea could they produce. A restricted plague epidemic that had been going on in Agra suddenly came to an end at the time of the disappearance of the fleas. On a previous occasion when plague was present in Agra (A.D. 1618), it appears to have come to an end at the same time of the year[1]. Tidswell noticed an analogous coincidence in Sydney[2]. He says, "It happened that whereas during the prevalence of plague, we had no particular difficulty in collecting the 100 specimens (of fleas) mentioned above, yet since the disappearance of the epizootic the rats examined have been remarkably free from fleas. Our frequent searches for specimens have been most usually fruitless. It was only now and then that we have come across two or three fleas on some particular rat. These rare specimens were either *Typhlopsylla musculi*, *Pulex fasciatus*, or *Pulex pallidus*." That is to say the fourth species of flea that Tidswell found on rats, the *Pulex serraticeps*, had vanished at the time of the cessation of plague in Sydney. It is noteworthy that this species of flea has been found in Sydney on cats, dogs, rats, human beings, and a wallaby.

J. Ashburton Thompson[3] from a study of the 1902 plague outbreak

[1] The Emperor Jehangir in his diary thus refers to this outbreak in Agra:—"During the last three years the disease has caused many deaths during the winter; but at the beginning of the summer it dies down to reappear at the first commencement of the cold weather." Jehangir mentions the mortality among rats. I am indebted for this reference to Colonel Lukis of the Indian Medical Service.

[2] "Report on Ectoparasites of the Rat," by Dr F. Tidswell, published in Ashburton Thompson's *Report on a Second Outbreak of Plague at Sydney in* 1902, p. 71.

[3] Ashburton Thompson's *Report on a Second Outbreak of Plague at Sydney in* 1902, p. 78.

in Sydney concludes that the flea must be able to communicate the virus "many hours, and even some days after it has received it." The facts brought forward in this paper suggest that in India the flea may retain the power of transmitting the disease for weeks or even months. Simond[1] has suggested that the retention of the infection by fleas may be the cause of recrudescences of the disease, which, as he shows, usually occur at the interval of a year after the first appearance of the outbreak.

Evidence of development of plague bacillus within body of flea.

As evidence that the plague microbe developes within the body of the flea, I can only quote the following observation of my own made during the above-mentioned plague outbreak in Agra. In April, 1901, a rat was brought to me that had been found dead in the grain dealers' quarter in Agra shortly after the first human case of the disease had occurred. No trace of the plague microbe could be found, either by microscopical examination or by culture in any of the tissues of the rat. On the rat, however, I found a living flea. This I caught and placed in a tube of sterile bouillon. The tube was violently shaken. The flea was then taken out and placed in a second tube of bouillon and similarly treated. The process was repeated several times, with the object of removing as far as possible saprophytic bacteria that might be present on the surface of the flea. After the above treatment the flea was dissected, with strict aseptic precautions, under a dissecting microscope. The stomach was taken out and cut in two pieces. One half was placed on agar-agar, and from it a pure culture of plague was obtained (as shown by involution forms on salt agar, &c.). The other half of the stomach was subjected to microscopic examination. The only microbes visible were bacilli with rounded ends identical in appearance with those of plague. These were arranged in clusters of about a dozen individuals each, and appeared to be embedded in the tissues of the stomach wall. No bacilli were observed in the liquid contents of the stomach. The arrangement of the bacilli in clusters obviously suggests that they were engaged in reproduction *in situ*.

Previous work on the subject.

Ogata (1897)[2] first found plague bacilli in fleas and suggested that these insects might be concerned in the spread of the disease.

The German Bombay Plague Commission (1897) found plague

[1] *Loc. cit.*

[2] "Ueber die Pestepidemie in Formosa." *Centralbl. f. Bacteriol.*, Vol. XXI. 1897, pp. 769–777.

bacilli in fleas, but did not consider that the bite of the flea was the means of transmission of the malady.

Simond[1] in 1898 observed bacilli identical in appearance with those of plague in the stomach contents of fleas from infected animals. He brought forward evidence tending to prove that such infected fleas could transmit the infection by biting. He also adduced epidemiological reasons for believing that this was the most frequent means of transmission of the disease.

In the same year, on epidemiological grounds, I suggested that some biting insect might be the means of the transmission of the disease from rats to men[2].

Nuttall[3], who has subjected earlier work on the relation of fleas to plague to a critical examination, suggests that possibly rat-fleas may attack men in plague epidemics, when their natural hosts are dying off rapidly in and about human dwellings (see Addendum, Note II.).

Thompson and Tidswell[4] in Australia have found that fleas from plague rats, when triturated and injected into susceptible animals could produce plague.

Galli-Valerio[5] has attacked the theory that plague can be conveyed from rats to men by fleas on the grounds that species of fleas found on rats do not bite men.

But Thompson in Australia and Tiraboschi[6] in Italy have since found *Pulex serraticeps* on rats, and it is known that this species of flea will bite man.

Tidswell has shown that three species of flea found on rats will bite man, namely, *P. serraticeps*, *P. fasciatus*, and *P. pallidus*.

Failure has frequently accompanied attempts to transmit plague to healthy rats by means of fleas coming from infected animals, as in the

[1] *Loc. cit.*

[2] Hankin, "La propagation de la Peste," *Annales de l'Institut Pasteur*, 1898, p. 705.

[3] Nuttall, "On the rôle of insects, arachnids, and myriapods as carriers in the spread of bacterial and parasitic diseases of man and animals. A critical and historical study." *Johns Hopkins Hospital Reports*, Vol. VIII. 1899, p. 21.

[4] Thompson and Tidswell, *Report on the Outbreak of Plague at Sydney*, 1900.

[5] Galli-Valerio, "Quelques observations sur la morphologie du Bacterium Pestis et sur la transmission de la Peste bubonique par les puces des rats et des souris." *Centralbl. f. Bacteriol.*, Vol. XXVIII. 1900, p. 842.

"The part played by the fleas of rats and mice in the transmission of Bubonic Plague," *Journal of Tropical Medicine*, Feb. 1902.

"Les nouvelles recherches sur l'action des puces des rats et des souris dans la transmission de la Peste bubonique." *Centralbl. f. Bacteriol.*, Vol. XXXIII. 1903, p. 753.

[6] Tiraboschi, "Beitrag zur Kenntniss der Pestepidemiologie. Ratten, Mäuse, und ihre Ektoparasiten." *Archiv für Hygiene*, Vol. XLVI. p. 251.

experiments of Tidswell[1] and Kolle[2]. Simond only succeeded in two experiments out of four. Simond has suggested that such failures are due to the fact that healthy rats can catch fleas. But in view of the considerations brought forward in this paper it is obvious that such experiments should be repeated, firstly, with different species of flea, secondly, regard being had to the possibility that the fleas may only become capable of transmitting the disease after the lapse of a period varying from ten to twenty days or more from the date of their exposure to the infection.

Chalmers, the Medical Officer of Health, in his Report on Plague in Glasgow in 1900 has adverted to the possibility of an insect (the flea) "acting as a temporary host of the bacillus of plague," and inoculating the disease by puncture, "after the manner in which the malarial parasite is transmitted through the bite of certain mosquitoes."

I owe my best thanks to Dr Nuttall, of Cambridge, for his advice and help while writing the latter portion of this paper.

ADDENDUM.

I. In "The Blot upon the Brain," by Dr W. W. Ireland (published in 1893), on p. 125, the author in the course of an attempt to prove the insanity of Mahommed Tughlak, states "We read of a great pestilence in 1341, well-nigh destroying a whole army in the Deccan. Was this an invading epidemic of the Black Death which so terribly thinned the population of Europe a few years later?" This remark of Dr Ireland's has only come to my knowledge after the first correction of my proofs.

II. I have just seen a newspaper report of a paper by Captain Liston, I.M.S., on the subject of "Plague, rats, and fleas," which contains important evidence bearing on the above views. The report is published in the *Times of India* for the 26th November 1904, and the paper will be published in full in the *Journal of the Bombay Natural History Society*. Captain Liston brings forward definite evidence of the truth of Nuttall's suggestion that rat-fleas may attack men after their natural hosts have died off. He finds that man rarely harbours the rat-flea under normal conditions. Out of 246 fleas caught on man, in the absence of plague, only one was a rat-flea. But during a plague outbreak in a lodging house (apparently in Bombay), out of 30 fleas caught on the human inhabitants no less than 14 were rat-fleas. Similar observations were made during a plague epidemic among guinea-pigs. Under normal conditions these animals are not infested by rat-fleas. But during the epidemic no less than 18 rat-fleas were found on one sick guinea-pig. Captain Liston also urges the particular infectivity of infected houses at night as "proof that the plague virus is transmitted by means of fleas." We at length seem to be in a position to understand the psalmist's phrase "the pestilence that walketh in darkness." The report of Captain Liston's paper only came into my possession on the 14th December, long after my own paper was sent to press.

[1] *Loc. cit.*

[2] Kolle, "Bericht über die Thätigkeit in der zu Studien über Pest eingerichteten Station des Instituts für Infectionskrankheiten." *Zeitschr. f. Hygiene*, Vol. XXXVI. 1901, p. 397.

AN IMPROVED METHOD OF CONSTRUCTING SHORTENED LIFE-TABLES FOR PUBLIC HEALTH COMPARATIVE STATISTICS.

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As is only too well known by those who have undertaken the task, the work of constructing a Life-Table by an extended method, even when "graphic" means are employed for lightening the labour, is long and wearisome.

When the tables setting forth numerical facts for every separate year of age are at length completed, although they are duly printed, no one, except for the purpose of minute criticism, pays much attention to them.

The practical outcome, both for author and for readers, is that attention is directed to comparative Tables in which the "numbers of survivors" and the "expectation of life" are given for certain ages at intervals of five or ten years. Although corresponding Tables of the fractions expressing the "chances of living a year" at certain ages are also given, these are scarcely necessary, as Tables of mean Death-rates in age-groups are more readily comprehended.

If, therefore, a method can be devised, by which, with but comparatively little labour, from the foundation figures of census enumerations and death records, a series of l_x and E_x values at interval of five years can be obtained, with close approximation to the results which would be obtained by an "extended" method, whether "analytical" or "graphic," all will be gained which is required for the purposes of Public Health.

The present writer has already done some work in this direction in so modifying the original short method of the late Dr Wm. Farr, as to

obtain fairly accurate E_x values. A description of the methods has already been published in this *Journal* (see Vol. II, No. 1).

These methods, however, are merely empirical and somewhat crude from a mathematical point of view, and they do not give, especially at the later ages, anything like true l_x values.

It is therefore now proposed to describe how by certain simple applications of exact mathematical principles a series of l_x and E_x values at intervals of five years from age 0 to age 85 may be obtained, with certainty that they will very nearly coincide with the corresponding values of the most laborious and accurate extended method which can be employed.

Instead of having to traverse one by one the successive yearly steps of the stairway of life, after the first five, the steps may be taken five at a time.

In order to limit the scope of this paper and to save needless repetition it is presumed at the outset that the reader is acquainted with, and has access to, papers previously published in this *Journal* in Nos. 1, 2, and 3, Vol. II, and also with the paper by Drs Newsholme and Stevenson in No. 3 of Vol. III.

It is therefore supposed that the preliminary work required in the construction of *all* Life-Tables has been completed, that is, Tables have been compiled of the "lives at risk" or "years of life," and of the deaths in each of the usual age-groups for the decennium being dealt with, and that p_x values have been calculated for each of the first five years of age, and by means of these l_x values from l_0 to l_5 inclusive, and then P_x values from P_0 to P_4 inclusive.

Preliminary remarks relating to the principles on which the methods of calculation to be described are based.

If reference be made to the two diagrams representing the curves of population and deaths which are given in the paper of Drs Newsholme and Stevenson above indicated between pp. 302—303, or to similar diagrams given in Dr Newsholme's *Vital Statistics*, 3rd edition, pp. 266—267, it will be evident that if an exactly corresponding point x be taken anywhere in the base-lines of the two curves, and the two ordinates at point x measured, there will be obtained the numbers of population and deaths respectively belonging to the exact age x (which may be indicated by the letters P and d), and the mean death-rate per unit, during the year of age of which x is the centre, which may be

denoted by m_x will be $\frac{d}{P}$, and the chance of living a year, existing at exact age x, denoted by $p_x{}'$ will be expressed by

$$\frac{2 - m_x}{2 + m_x} \text{ or by } \frac{2P - d}{2P + d}.$$

It will also be evident that if the original data had been arranged for each age-group in the form of twice population minus deaths and twice population plus deaths, two other curves might have been drawn through parallelograms first constructed, and that the measurement of the two ordinates at a corresponding point x in the base-lines of the two curves would at once give the numerator and denominator of the $p_x{}'$ fraction, viz. $\frac{2P - d}{2P + d}$.

In the methods of working to be now described, instead of drawing a curve by a "graphic" process, and measuring the ordinates required to be interpolated from this curve, each required ordinate is calculated

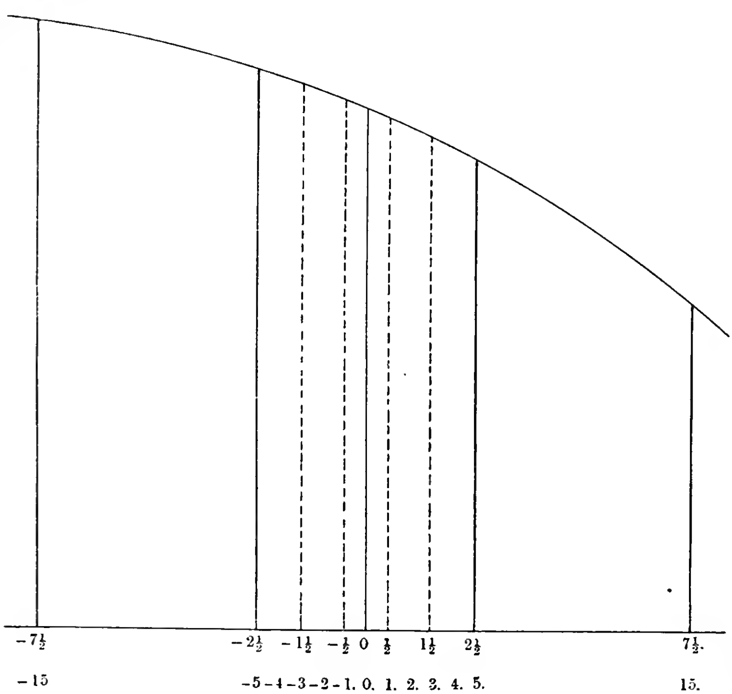

Fig. 1.

by a formula which expresses its value in terms of the ordinates which are given as the foundation series of the curve. Now when these given ordinates are at equal distances apart, and only four or five are taken in a series (that is, when interpolation is effected by only three or four orders of differences), and when only centrally situated ordinates, or sums of ordinates symmetrically arranged with regard to the central point of the base-line, are required, the formulae can be reduced to extremely simple forms. Thus (see Fig. 1) if four ordinates are given separated by 10 units of measurement (years) in the base-line, which may be denoted respectively by the symbols u_{-15}, u_{-5}, u_5, u_{15}, the central ordinate u_0 is measured by the formula

$$u_0 = \frac{9\,(u_{-5} + u_5) - (u_{-15} + u_{15})}{16}.$$

This simple calculation can certainly be effected in less time than would be required to draw a curve and measure the required ordinate.

In arranging, however, for the interpolation of ordinates, so as to measure by calculation the numbers of population and deaths separately, or combined as $2P - d$ and $2P + d$, as existing at certain *exact ages*, since the foundation figures are given in groups of ages it is necessary to reconstruct these figures in a form giving the numbers at each age and upwards, so that each given ordinate may be a "linear quantity."

Thus in Fig. 2 we have given five equidistant ordinates separated by five-yearly intervals, measuring respectively $2P - d$ or $2P + d$ at age 5 and upwards, at age 10 and upwards, and so on to age 25 and upwards. Now if u_{14} be interpolated, then $u_{14} - u_{15}$ will give the numbers belonging to the year of age 14 to 15, and similarly if u_{16} be interpolated, $u_{15} - u_{16}$ will give the numbers belonging to the year of age 15 to 16, &c.

But it would be very tedious and laborious to calculate $\frac{2P-d}{2P+d}$ for every year of age by this method.

There is, however, a simple way of measuring the values of $2P - d$ and $2P + d$ belonging to *exact age* 15 (*i.e.* age 0 in the given series), the formula for which is arrived at by the differential calculus, as it has been applied by Mr A. C. Waters.

Thus $2P - d$, or $2P + d$, at exact age 0

$$= -\frac{8\,(u_{-5} - u_5) - (u_{-10} - u_{10})}{60},$$

and p_0' (that is, the chance of living a year which exists at exact age 0)

$$= \frac{2P-d}{2P+d} \text{ at exact age } 0.$$

This formula is true when the ordinates represent *numbers*, but as will now be explained it has to be modified when the ordinates in Fig. 2 represent the *logarithms* of $2P-d$ and $2P+d$ at age x and upwards.

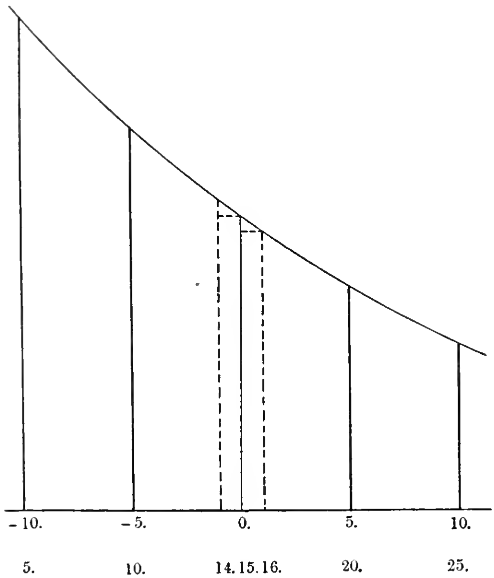

Fig. 2.

When this is the case, as is required in actual working, if the symbol u_x be used to denote the log of $2P-d$ at age x and upwards, and the symbol U_x to denote the log of $2P+d$ at age x and upwards, then it can be shown that

$$\log p_0' = \{u_0 + \log [8(u_{-5} - u_5) - (u_{-10} - u_{10})]\}$$
$$- \{U_0 + \log [8(U_{-5} - U_5) - (U_{-10} - U_{10})]\}.$$

If the foundation series of u_x and U_x values be completed at five-yearly intervals by simple formulae of interpolation, it is then possible to readily obtain by the formula just given a complete series of $\log p_x'$ values at five-yearly intervals.

These values may be considered as ordinates of a curve (the p_x curve), and the series may be completed at yearly intervals by formulae of interpolation. In No. 2 of Vol. II. of this *Journal* it has also been shown how by a graphic process applied to the given $\log p_x'$ values, the intermediate values may be obtained by measurement. Thus if (see Fig. 1) in the series at five-yearly intervals, viz. $-7\frac{1}{2}$, $-2\frac{1}{2}$, $2\frac{1}{2}$ and $7\frac{1}{2}$, we

obtain the sum of the logs at -2, -1, 0, 1 and 2, we can pass by one step of addition from $\log l_{-2\frac{1}{2}}$ to $\log l_{2\frac{1}{2}}$.

However, it is possible with still greater ease to obtain a truer value by a single calculation. Since any number of ordinates may be interpolated between $-2\frac{1}{2}$ and $2\frac{1}{2}$, each having as good a claim as the other to represent the true $\log p_x'$ value *at its own point*, it is obvious that if we wish to obtain the true mean $\log p_x$ value between ages $-2\frac{1}{2}$ and $2\frac{1}{2}$ the greater the number of intermediate ordinates interpolated the nearer to the true value will be the resulting mean. Thus

(1) If (as shown by the dotted lines in Fig. 1) *four* intermediate equidistant ordinates are interpolated by calculation at yearly intervals, the mean of the six ordinates $-2\frac{1}{2}$, $-1\frac{1}{2}$, $-\frac{1}{2}$, $\frac{1}{2}$, $1\frac{1}{2}$, and $2\frac{1}{2}$, can be shown to be equal to

$$\frac{12{\cdot}8\,(u_{-2\frac{1}{2}} + u_{2\frac{1}{2}}) - {\cdot}8\,(u_{-7\frac{1}{2}} + u_{7\frac{1}{2}})}{24}.$$

(In this and the succeeding formulae u_x means $\log p_x'$.)

(2) If *nine* intermediate ordinates, at intervals of half a year, be interpolated, the mean of the 11 ordinates

$$= \frac{12{\cdot}9\,(u_{-2\frac{1}{2}} + u_{2\frac{1}{2}}) - {\cdot}9\,(u_{-7\frac{1}{2}} + u_{7\frac{1}{2}})}{24}.$$

(3) If *nineteen* intermediate ordinates, at intervals of $\frac{1}{4}$ year, be interpolated, the mean of the 21 ordinates

$$= \frac{12{\cdot}95\,(u_{-2\frac{1}{2}} + u_{2\frac{1}{2}}) - {\cdot}95\,(u_{-7\frac{1}{2}} + u_{7\frac{1}{2}})}{24}.$$

(4) On increasing the number of intermediate ordinates more and more, the mean value would be found to approximate more and more closely to

$$\frac{13\,(u_{-2\frac{1}{2}} + u_{2\frac{1}{2}}) - 1\,(u_{-7\frac{1}{2}} + u_{7\frac{1}{2}})}{24}.$$

On multiplying the above value by 5, corresponding to the five separate yearly units of interval, the coefficients in the numerator become 65 and -5, and then, multiplying both the numerator and the denominator of the expression by 2, the result is reduced to the convenient working formula

$$\frac{130\,(u_{-2\frac{1}{2}} + u_{2\frac{1}{2}}) - 10\,(u_{-7\frac{1}{2}} + u_{7\frac{1}{2}})}{48}.$$

The above formula is *one* application of a general formula worked out by the integral calculus, viz.

$$\int_{-n}^{n} u_x dx = \frac{n}{3}\left[\frac{13(u_{-1}+u_1)-(u_{-3}+u_3)}{4}\right].$$

It is thus possible in this extremely simple way to obtain the logs which by successive steps of addition enable us to pass from log l_x to log l_{x+5}.

After having obtained the complete series of log l_x values and taken out their corresponding numerical values, these are also to be considered ordinates of a continuous curve (the l_x curve), and (see Fig. 1), taking u_x as denoting l_x, the sum of the years of life lived by $l_{-2\frac{1}{2}}$ persons in the interval from age $-2\frac{1}{2}$ to age $2\frac{1}{2}$ is obtained by the formula

$$\frac{130(u_{-2\frac{1}{2}}+u_{2\frac{1}{2}})-10(u_{-7\frac{1}{2}}+u_{7\frac{1}{2}})}{48}.$$

Construction of the Shortened Life-Table.

In the actual process a working-sheet with successive series of columns will be used. It is proposed to explain in order how the respective columns are to be constructed.

Columns 1 *and* 2, *headed* u_x *and* U_x.

It is first of all necessary to put the foundation figures of "lives at risk" and deaths into the form of $2P-d$ and $2P+d$ at age x and upwards. The lowest age-group being at age 85 and upwards, the addition of $2P-d$ and $2P+d$ for ages 75—85 will give the number for age 75 and upwards, and so on until the figures for age 5 and upwards are arrived at.

From the *numbers* are then to be derived the corresponding *logarithms* so that No. 1 column will contain the logs of $2P-d$ at age x and upwards, the given series being for ages 5, 10, 15, 20, 25, 35, 45, 55, 65, 75, and 85. This is headed u_x. Similarly the second column U_x is obtained by setting down for the same series of ages the logs of $2P+d$ at age x and upwards.

These columns have then to be completed at five-yearly intervals by interpolations, the formulae for which are as follows, beginning at the top of the series:

$$u_0 = 5(u_5 - u_{20}) + u_{25} - 10(u_{10} - u_{15}),$$

$$u_{30} = \frac{5(u_{15}+9u_{25}+3u_{35})-(24u_{20}+u_{45})}{40},$$

$$u_{40}=\frac{9(u_{35}+u_{45})-(u_{25}+u_{55})}{16}=\frac{10(u_{35}+u_{45})-(u_{25}+u_{35}+u_{45}+u_{55})}{16},$$

$$u_{50}=\frac{9(u_{45}+u_{55})-(u_{35}+u_{65})}{16},$$

$$u_{60}=\frac{9(u_{55}+u_{65})-(u_{45}+u_{75})}{16},$$

$$u_{70}=\frac{3(u_{45}+30u_{65}+20u_{75})-5(4u_{55}+u_{85})}{128},$$

$$u_{80}=\frac{5(3u_{65}+9u_{75}+u_{85})-(u_{55}+40u_{70})}{24},$$

$$u_{90}=u_{65}+10(u_{75}-u_{80})-5(u_{70}-u_{85}),$$

$$u_{95}=u_{70}+10(u_{80}-u_{85})-5(u_{75}-u_{90}).$$

(Similar formulae of course apply to the U_x values in column 2.)

It is desirable to check the interpolations of u_{70}, u_{80}, u_{90} and u_{95} by finding that the last term coincides with the value obtained by

$$u_{95}=u_{45}+10(u_{65}-u_{75})-5(u_{55}-u_{85}).$$

The series from u_{65} to u_{95} inclusive should have a constant fourth difference.

Column 3, headed $\log p_x'$.

From the logs in columns 1 and 2 it is now possible to obtain a series of $\log p_x'$ values from $\log p_{10}'$ to $\log p_{85}'$ inclusive by the formula previously given.

Each $\log p_x'$ value is to be derived from a series of five equidistant u_x and U_x values of which the central term in each series is taken as u_0 and U_0 respectively.

Thus, to take as an illustration the calculation of $\log p_{10}'$ from the data of the Brighton Life-Table for 1898—1900 (males).

	x	u_x	U_x
−10	0	6·1011849	6·1072497
−5	5	6·0543709	6·0604667
0	10	5·9956540	6·0024205
5	15	5·9296871	5·9374090
10	20	5·8601732	5·8689494

$8(u_5-u_{15})-(u_0-u_{20})=0·7564587$

$\log ·7564587=\bar{1}·8787852$

$8(U_5-U_{15})-(U_0-U_{20})=0·7461613$

$\log ·7461613=\bar{1}·8728328$

$5·9956540+\bar{1}·8787852=5·8744392$

$6·0024205+\bar{1}·8728328=5·8752533$

$5·8744392-5·8752533=\bar{1}·9991859=\log p_{10}'$.

This column has to be completed by differencing the series of log p_x' values for ages 65, 70, 75, 80 and 85, and by carrying down the differences values may be obtained for ages 90, 95, 100, 105, and 110.

Column 4, headed $\int_x^{x+5} log\ p_x$.

In the first place it must be noted that for the age-periods 5—10 and 10—15 the required values are to be simply obtained from the "lives at risk" and total deaths for the respective age-groups 5—10 and 10—15 by the fraction $\left(\frac{2P-d}{2P+d}\right)^5$, that is the log is obtained by

$$[(\log 2P-d)-(\log 2P+d)]\times 5.$$

It has been found by repeated trials involving much more complicated calculations than those set forth in this paper that the values obtained by the above indicated simple method are the best for the present purpose. However, the value for the age-period 5—10 thus obtained is a little less than the true value.

These values may be therefore obtained first and set down in column 4.

The first value to be obtained by the method of "integration" which has been already described, is the log p_x value between ages 15 and 20.

To take an illustrative case again from the Brighton Life-Table.

	Ages	log p_x'
$-7\frac{1}{2}$	10	$\bar{1}$·9991859
$-2\frac{1}{2}$	15	$\bar{1}$·9988287
$2\frac{1}{2}$	20	$\bar{1}$·9980144
$7\frac{1}{2}$	25	$\bar{1}$·9974963

The formula being $\dfrac{130\,(u_{-2\frac{1}{2}}+u_{2\frac{1}{2}})-10\,(u_{-7\frac{1}{2}}+u_{7\frac{1}{2}})}{48}$.

	$u_{-2\frac{1}{2}}+u_{2\frac{1}{2}}$	$u_{-7\frac{1}{2}}+u_{7\frac{1}{2}}$
	$\bar{1}$·9988287	$\bar{1}$·9991859
	$+\bar{1}$·9980144	$+\bar{1}$·9974963
	$\bar{1}$·9968431	$\bar{1}$·9976822
taking the differences from 0, or the co-logs ...	0·0031569	0·0033178
	×130	
	947070	
	31569	
	·4103970	
	− 331780	

0·3772190÷48=0·0078587

∴ required log=$\bar{1}$·9921413

This series of calculations is to be carried on until the value between ages 100 and 105 is obtained.

Column 5, *headed log* l_x.

Commencing with $\log l_5$ by successive addition of the logs in the preceding column, the values are obtained for this column from $\log l_{10}$ to $\log l_{105}$.

Column 6, *headed* l_x.

This column is simply obtained by taking out the numerical values of the logs in the preceding column. An additional term l_0 must be interpolated at the top of the column by the formula

$$l_0 = 4\,(l_5 + l_{15}) - (6l_{10} + l_{20}).$$

This, of course, has no relation to the *true* l_0 value, but is merely required for the purpose of calculating the value of $P_{5\text{ to }10}$ for the succeeding column.

Column 7, *headed* $P_{x\text{ to }x+5}$, or $Q_x - Q_{x+5}$.

In this column the years of life lived by l_x persons in the interval from age x to age $x+5$ are set down.

The simple formula required is identical with that already used for column 4,

$$\frac{130\,(l_{-2\frac{1}{2}} + l_{2\frac{1}{2}}) - 10\,(l_{-7\frac{1}{2}} + l_{7\frac{1}{2}})}{48}.$$

Taking an illustrative case from the Brighton Life-Table.

	x	l_x
$-7\frac{1}{2}$	0	78541
$-2\frac{1}{2}$	5	75970
$2\frac{1}{2}$	10	74549
$7\frac{1}{2}$	15	73784

$$\begin{array}{rr}
75790 & 78541 \\
+74549 & +73784 \\
\hline
150339 & 152325 \\
\times 130 & \\
\hline
4510170 & \\
150339 & \\
\hline
19544070 & \\
-\ 1523250 & \\
\hline
18020820 \div 48 = 375434 &
\end{array}$$

This formula has to be used as far as the interval between age 90 and age 95.

The years of life lived after age 95 are to be obtained by the simpler integration formula

$$\frac{5\,(l_{95}+4l_{100}+l_{105})}{3}.$$

Column 8, headed Q_x.

The value obtained by the last given formula is to be set down as Q_{95}, then by successive additions from below upwards the Q_x values are obtained as far as Q_5.

In order to obtain Q_0 it is simply necessary to add to Q_5 the sum of P_0, P_1, P_2, P_3 and P_4 already obtained.

Column 9, headed E_x.

This is to be obtained from age 0 to age 85 by the formula $E_x=\dfrac{Q_x}{l_x}$.

Column 10, headed $E_{x \text{ to } x+n}$.

It is desirable for the sake of being able to obtain those useful and interesting applications of a Life-Table which are related to the term "Life-capital" to have values expressing the mean expectation of life in age-groups. This can be readily accomplished—

(1) By successively adding to the Q_5 of the shortened Life-Table the values of P_4, P_3, P_2, P_1 and P_0 the corresponding Q_x values are obtained, then

$$E_{0-5}=\frac{Q_0+Q_1+Q_2+Q_3+Q_4}{P_0+P_1+P_2+P_3+P_4}-\tfrac{1}{2}.$$

Before proceeding to the next value E_{5-10} it is necessary to calculate a hypothetical Q_0 value (which must not be confounded with the *true* Q_0 value) by the formula $Q_0=4\,(Q_5+Q_{15})-(6Q_{10}+Q_{20})$.

Then (the l_0 value being the previously *calculated* one),

(2) $$E_{5-10}=\frac{13\,(Q_5+Q_{10})-(Q_0+Q_{15})}{13\,(l_5+l_{10})-(l_0+l_{15})}.$$

A similar formula is to be used for the values of E_{10-15}, E_{15-20} and E_{20-25}.

(3) $$E_{25-35}=\frac{Q_{25}+4Q_{30}+Q_{35}}{l_{25}+4l_{30}+l_{35}}.$$

A similar formula will give the values as far as E_{75-85}.

(4) $$E_{85-105}=\frac{(Q_{85}+4Q_{90}+Q_{95})+(l_{95}+4l_{100}+l_{105})}{(l_{85}+4l_{90}+l_{95})+(l_{95}+4l_{100}+l_{105})}.$$

Comparison of the results obtained by the shortened method just described, with the corresponding figures of four extended Life-Tables.

The methods of calculation which have been described have been applied to the data of four extended Life-Tables:

(1) The London Life-Table (for males) based on the experience of 1891—1900.

This was calculated by a very laborious extended method which had been previously suggested by the present writer in a paper contributed to the *Journal of the Royal Statistical Society*, Vol. LXII. Parts 3 and 4.

By elaborate processes of interpolation values of $2P - d$ and $2P + d$ were calculated for every separate year of age, and then the yearly p_x values by $\frac{2P-d}{2P+d}$.

(2) England and Wales (males) 1891—1900.

No official Life-Table has as yet been issued, but in addition to other Life-Tables which have been published by the writer, another has been specially prepared for the purpose of this paper, by a method not quite identical with that of the London Life-Table, but essentially similar, in that values of $2P - d$ and $2P + d$ have been interpolated all throughout in series with five orders of differences. The exact details of the method are given in a lecture by the writer, which forms one of a series of advanced Public Health Lectures given in 1903–4 under the auspices of the Victoria University of Manchester, and published by Sherratt and Hughes at the University Press, Manchester (see pp. 16—19, "Method IV.").

(3) The second Life-Table for Brighton of Dr Newsholme for 1891—1900 (males).

This was constructed by means of the "graphic" method which Dr Newsholme has so ably expounded and advocated.

(4) A Life-Table for Scotland (males) based on the experience of 1891—1900 by Mr T. Adam, M.A., &c., published in the *Journal of the Royal Statistical Society*, Vol. LXVII., Part 3, Sept. 1904.

This also was calculated by the "graphic" method as described by Dr Newsholme.

In order to economise space it is only proposed to give two tables setting forth the differences from the values of the respective extended Life-Tables of the corresponding values obtained by the shortened method.

(1) *Differences of l_x values*—100,000 *at birth.*

Males.

Age	England and Wales	London	Brighton	Scotland
0	0	0	0	0
5	0	0	0	0
10	− 2	−20	− 1	− 8
15	− 2	−19	− 1	− 7
20	+36	−18	+ 31	+ 39
25	+50	+47	+ 51	+ 70
30	+76	−37	+ 64	+ 17
35	− 2	+ 1	+ 16	+ 10
40	+14	+56	+ 32	+ 19
45	+22	+31	− 2	+ 12
50	+14	+27	+ 19	− 14
55	+23	+35	− 14	− 15
60	+52	+55	+ 10	+140
65	+71	+85	+ 44	− 31
70	+57	+68	+128	−167
75	+32	+28	− 15	+ 63
80	+ 7	+ 5	− 27	+203
85	− 2	+ 2	+100	−146

(2) *Differences of E_x values.*

Males.

Age	England and Wales	London	Brighton	Scotland
0	+0·03	+0·03	+0·03	+0·01
5	+0·04	+0·04	+0·06	+0·01
10	+0·04	+0·04	+0·07	+0·02
15	+0·04	+0·05	+0·07	+0·02
20	+0·02	−0·01	+0·05	±0·00
25	+0·01	+0·01	+0·03	−0·02
30	±0·00	+0·05	+0·03	±0·00
35	+0·03	+0·03	+0·05	+0·01
40	+0·02	+0·01	+0·04	±0·00
45	+0·01	+0·02	+0·06	+0·01
50	+0·02	+0·02	+0·06	+0·02
55	+0·02	+0·01	+0·08	+0·02
60	+0·01	+0·01	+0·08	−0·04
65	+0·01	−0·01	+0·08	+0·01
70	±0·00	−0·01	+0·06	+0·08
75	+0·01	±0·00	+0·15	+0·03
80	±0·00	±0·00	+0·30	−0·12
85	±0·00	−0·02	+0·58	+0·12

The results given in the above tables may almost be left to speak for themselves.

When the results of the short method are compared with those of the two elaborately constructed extended Life-Tables for England and Wales and for London the closeness of approximation is remarkable both as regards l_x and E_x values, but especially the latter, and even the differences of the l_x numbers, to any one who has by experiment found out how wide is the range of differences in the l_x values obtainable from the same data by different extended methods, will appear relatively insignificant.

Such differences, small as they are, as do exist in the E_x values are practically wholly due to differences in the p_x values and therefore in the l_x values, because it has been found in both instances that the simple method of integrating l_x values given at five-yearly intervals *when applied to the numbers of the respective extended Life-Tables* gives coincident E_x values, with the exception of a few differences of $\pm 0{\cdot}01$ or $\pm 0{\cdot}02$.

The comparison of the results of the short method with those of the two extended Life-Tables constructed by the "graphic" method is also striking as regards the degree of closeness of approximation until the later ages are arrived at.

(1) One inference which may be drawn is that certainly until about the age of 65 the results of the graphic method correspond closely with those obtainable by the most minutely accurate analytical method.

(2) Another possible inference which the writer would suggest is only an extension of what has been already admitted by the advocates of the "graphic" method, viz. that the unreliability and want of accuracy in the results of this method which exist at ages *after* 85, really begin *before* this age is reached, and that it might be better to commence to use the method of differencing the logs of p_x values (*not* the numerical values of the logs) after age 65, certainly not later than age 75.

A simple method of effecting this has been described in this *Journal*, Vol. III., No. 3, pp. 348, 349.

The discrepancies in the E_x results of the Brighton Life-Table are due to the fact that the value of log p_{85} (see Vol. III., No. 3, p. 308 of this *Journal*) is much lower than it is found to be by the more exact application of an analytical method. This has meant that the succeeding values of p_x are too low and the value of E_{85} has been made 3 years

instead of about $3\frac{1}{2}$ years. The deficiency in Q_{85} has been enough to lower the E_x values above, even to making E_0 too little by 0·02.

If this correction were made the comparative Table of E_x values would be as close as the results of the other instances.

(3) Another inference which may perhaps be drawn by some readers is that the results obtainable by the shortened method, which it has been the object of this paper to describe, are sufficiently accurate to render it a reliable instrument of statistical work, and to dispense with the trouble of using any extended method whether analytical or graphic.

In conclusion it may be stated that the simplicity and ease of the method have been only arrived at by devious wanderings in the mazes of methods much more complex and difficult.

Corresponding tables for females have also been worked out in all four instances with results equally satisfactory.

However, in the case of England and Wales the comparison has had to be made not with the results of the extended method as used for males, but with the results of a more elaborate shortened method, involving the use of five or six orders of differences, which in the case of males had been found to give results almost coinciding with those of the extended method.

As the object of this paper has been to simplify to the utmost possible extent, the more elaborate shortened method has been abandoned in favour of the simpler method as above described, seeing that the results obtained by this method are sufficiently accurate for all practical purposes.

FURTHER OBSERVATIONS ON A LEPROSY-LIKE DISEASE OF THE RAT.

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THE object of this paper is to record briefly the result of certain observations and experiments on a disease of the rat, which is chiefly interesting from the close resemblance it bears to leprosy in the human subject. Not only are the lesions similar in their macroscopical and microscopical appearances, but the causal bacillus closely resembles the *Bacillus leprae* in its morphological characters, distribution in the tissues, staining reactions, and refusal to adopt a saprophytic habit of life.

A large number of acid-fast organisms have been isolated and studied within the past few years; from smegma, dung, various grasses, milk, butter, and nasal mucus, etc. However, only a few organisms belonging to this group have been observed which bear a causal relation to naturally occurring diseases among animals. Indeed these may be limited to *B. tuberculosis*, the bacillus of Verruga of Peru, *B. leprae*, and certain acid-fast organisms of the streptothrix group giving rise to somewhat ill-defined diseases.

Until this condition in the rat was observed no disease was known in any of the lower animals which pathologically or bacteriologically had any very close resemblance to leprosy in man.

The disease was first observed by Stefansky (1903), while working at plague in Odessa. He found that 5 % of the rats (*Mus decumanus*) destroyed suffered from a condition affecting the skin and underlying muscles, and the lymphatic glands. Associated with the lesions, and apparently the causal agent, was a bacillus belonging to the acid-fast group. Stefansky gave an excellent description of the chief features of the disease. Rabinowitsch (1903), who had been working at Odessa during the time that Stefansky made his observations, on returning to Berlin was able to confirm Stefansky's results, for the rats there.

The disease had been observed in England and its nature recognised by the writer (1903) before the appearance of Stefansky's paper, and the experiments commenced in February, 1903, have been continued up to the present time. Since the first case was noted here, six other well marked examples of the disease, as it occurs naturally in the rat, have come under observation. Stefansky, Rabinowitsch, and the writer all stated that the animals which had been inoculated up to the time of their reports showed no signs of having acquired the disease, but since that time the writer has succeeded in obtaining a number of positive inoculation results in the case of rats.

The Disease as it occurs naturally.

Stefansky pointed out that there were two chief types of the disease, the one affecting primarily the skin and musculature, and the other confined to the lymphatic glands. There is, however, no very strict line of demarcation between the two types.

Skin and Muscle Lesions.

Patches of alopecia are the most marked feature. These may be extensive, affecting in some cases almost the whole thorax and abdomen. Above the surface of these bare patches of skin, elevated bosses, or even nodules, the size of a bean are frequently present. The surface of these elevations may be ulcerated, usually at the most prominent point. Ulceration is not, however, an essential feature and appears to result in part at least from mechanical causes (Fig. 1, Plate VI).

On incising an area affected in this manner the skin appears greatly thickened (up to half an inch), due to the presence of a pale yellow infiltration which cuts with a clean, dry, cheese-like surface. In most cases there is little tendency to softening and breaking down of the material. A scraping from its surface, stained by Ziehl-Neelsen's method, shows enormous masses of bacilli, many of them lying within the cells. Microscopical examination of a section through the skin and subjacent tissues reveals appearances which are almost identical with those found in the skin in lepra. The epithelial layers of the skin are but little affected; a few bacilli may be found to have forced their way between, or even into, the epithelial cells. The subepithelial infiltration is found to consist of cells lying in a stroma of connective tissue. The cells in part consist of leucocytes of different forms including lymphocytes, and in part of connective tissue cells; the endothelial cells

taking a prominent part in the formation of this granulation tissue. These cells are round, oval, or polygonal with a large nucleus and abundant protoplasm.

In a section appropriately stained one is struck by the presence of an enormous number of acid-fast bacilli with which nearly the whole of the cells of the tissue are packed (Fig. 5, Plate VII). The same difficulty in deciding whether the groups of bacilli lie in the lymphatic spaces is found as in the case of leprosy, but some of the appearances observed can be most satisfactorily interpreted in this way.

There is no doubt, however, that the majority of the bacilli actually lie within cells, and where these cells are of the large endothelial type an appearance strikingly resembling lepra cells results. The sebaceous glands and hair roots have mostly disappeared; those remaining appear to be undergoing a process of atrophy.

In the subcutaneous connective tissue the most prominent feature is the disappearance of fat. The whole region is infiltrated with cells, for the most part filled with bacilli. Here and there are small rounded or oval areas in which all appearance of cellular structure has disappeared; only *débris*, fragments of cell nuclei, and enormous masses of bacilli being visible. In one case in which the mammary gland was well developed the connective tissue framework and lymphatics were extensively invaded by the bacilli, but none of these could be found within the acini or ducts.

An interesting feature of the disease is the manner in which the striated muscle is invaded by the bacilli. Between the muscle fibres are frequently seen groups of cells of the types already mentioned. As these foci increase in size they cause atrophy of the muscle fibres and at the same time some of the cells burst and the bacilli penetrate the muscle fibre. In the early stage the bacilli are seen lying in the fibre, later this becomes degenerated, loses its striation, and as the invasion progresses only its outline is indicated by a tightly packed mass of bacilli round the nucleus. Even the outline may be lost by fragments of muscle fibre becoming more or less spindle-shaped and thus presenting the appearance of large cells.

Lymphatic Gland Lesions.

The lesions next in importance to those of the skin occur in the lymphatic glands. The glandular enlargement may be widespread; the axillary, cervical, and inguinal glands may all be affected, or only one of these groups. The enlargement may be considerable (Fig. 2,

Plate VI). In one case, in which most of the glands were involved, some of these had reached the size of a small hazel-nut. On section of the glands there are neither nodules nor necrotic areas visible to the naked eye.

Microscopical examination. The capsule may be thickened and may contain great masses of bacilli. The chief invasion of the gland itself appears to be along the sinuses, but where the disease is advanced the whole gland may be invaded. Along the sinuses there are large numbers of cells apparently of endothelial origin, rounded, oval, or polygonal, with abundant protoplasm and large nucleus. The whole of the protoplasm is packed with bacilli, and a few organisms are also found lying free (Fig. 3, Plate VII).

Giant cells are numerous and occasionally of enormous size, the transverse diameter reaching 70—80μ, and containing many nuclei which may be situated either in the centre or at the periphery of the cell. They are sometimes vacuolated. The protoplasm of these cells is almost entirely occupied by masses of bacilli. They form beautiful objects either in smear preparations of glands or in sections (Fig. 2, Plate VII). Their mode of origin is difficult to determine just as in the case of lepra. It seems probable that they are greatly enlarged endothelial cells in which multiplication of the nuclei has taken place. The view, however, may be held that they are small lymphatic vessels which have been blocked by bacilli and that the nuclei are those of the endothelium originally lining the channel. The continuity of the cell outline is retained till a great size is reached, but in certain cases the cells seem to have become disintegrated from the overgrowth of bacilli.

Other Lesions.

When the cervical lymphatic glands were greatly enlarged an invasion of the neighbouring structures such as the submaxillary salivary glands was observed. Visceral lesions are rare—only in one case in a naturally infected animal has a small necrotic area containing the bacilli been found in the liver. The bone-marrow was found to be invaded.

An acid-fast bacillus was found in two cases in the nasal secretions, probably identical with that causing the lesions. This is interesting in relation to Stricker's (1899) observation of the frequency of the *Bacillus leprae* in the nasal mucus.

In one rat the loss of several toes was observed, and in others part of the tail had disappeared. As the writer has observed that bacilli

sometimes invade the nerve-trunks these losses may be connected with nerve changes. The lesions may, however, have been the result of trauma and not of disease.

The health of the diseased animals is sometimes seriously affected, and emaciation is a marked feature. Some of the animals were captured in broad daylight wandering outside their holes in a dazed condition.

The disease is evidently of wide distribution since it has been observed in Odessa, Berlin, and England. Six of the seven well-marked examples of the disease which have been examined were obtained within a few miles of Elstree, Herts.; the seventh was found in Norfolk.

The Bacilli.

The bacilli are straight or bent rods with round, or more rarely pointed ends, about $0{\cdot}5\,\mu$ broad and for the most part from 3 to $5\,\mu$ in length, though sometimes longer forms are observed. They may have a beaded appearance, the beads being few and large, and causing a bulging of the rod. The bacilli are strongly acid- and alcohol-fast. They stain readily with carbol-fuchsin, and resist the action of 25 % sulphuric acid or 3 % hydrochloric acid in alcohol, or 2 % anilin-hydrochloride—followed by alcohol. They stain by Gram's and Claudius' methods.

An interesting point about the staining of the bacillus which it has in common with that of leprosy is that if mounted in even slightly acid Canada balsam it gradually loses its bright red colour.

Cultivation experiments.

All attempts at cultivation of the bacillus in its acid-fast form have entirely failed. A large number of tubes containing all the ordinary laboratory media have been inoculated and kept under aerobic and anaerobic conditions but no growth took place. Special media have also been tried, such as a medium made from rat's flesh, and a medium with the addition of rat's blood-serum, but these have also failed to give a growth of the bacillus. Many pieces of tissues swarming with bacilli which have been left for over a year on appropriate media still show the bacilli staining well but no growth has taken place. As Rabinowitsch has pointed out, this makes it improbable that the bacillus is one of the known earth or dung bacilli, because these are easily cultivated and appear to be innocuous to rats. The writer has succeeded in obtaining from two of the affected rats cultivations of a diphtheroid bacillus.

This observation is of interest because a diphtheroid bacillus has been isolated by Babes and a number of observers from cases of lepra.

In his later publications Babes (1899) states that he has isolated this organism from twelve cases. Spronck (1898) isolated the same organism and found that it was agglutinated by the blood-serum of leprous patients, though not more so in certain cases than by the serum of non-leprous persons. As is remarked by Babes, a positive agglutination test would not prove the identity of this diphtheroid bacillus with the lepra bacillus, since if the bacillus is frequently associated with the acid-fast bacillus in cases of lepra it would naturally give rise to an agglutinating serum in these patients. The identity of the two bacilli would be demonstrated in a satisfactory manner only (1) if the diphtheroid bacillus could be so cultivated as to become acid-fast, (2) if it were demonstrated to have specific properties in relation to lepra such as the production of a specific tuberculin-like reaction, or (3) if it was found capable of producing lepra. Babes has carried out experiments in certain of these directions without, however, obtaining conclusive results.

Levy (1897 and 1899), Czaplewski (1898), Barannikow (1899), Teich (1899), and Kedrowski (1901) have all obtained cultures of bacilli resembling those of Babes. Kedrowski (1904) believes that he has obtained lesions resembling those of lepra by injecting his bacillus into rabbits.

The diphtheroid bacillus, isolated by the writer from rats, resembles the organism isolated by these workers, but its significance is open to the same criticism since, so far, it has been found impossible to convert it into the acid-fast one. There was no evidence of the acquisition of acid-fast properties by the diphtheroid bacillus after a sojourn of three months in the peritoneal cavity of the rat.

The only observation that makes it possible that the two organisms are in any way related will be found under the head of agglutination. A probable explanation of the presence of diphtheroid bacilli in lepra, and it may be also in the rat disease, is the fact that diphtheroid bacilli appear frequently to have their habitat in sebaceous glands, and in a weakened state of the skin they may be able to penetrate it and grow in the tissue.

Brief description of the main characters of the diphtheroid bacillus which was isolated from two of the diseased rats.

Morphology. In young cultures (24 hours old, on agar, or blood serum) it has the form of a small diplo-bacillus, or is sometimes very

like a Hofmann's bacillus. On the second or third day it becomes longer and has all the appearances of a typical diphtheria bacillus with segmentation, club-shaped ends, etc., and later it may form short, much segmented and branching filaments. In a two days' old culture, the club-shaped forms become very large, much segmented, and recall those of the *B. xerosis*.

Cultures. On solidified blood serum the growth is indistinguishable from that of the Klebs-Loeffler bacillus. On neutral agar and glycerine agar the growth is slower, the colonies are more delicate and transparent than those of the ordinary diphtheria bacillus. On neutral and alkaline broth it forms no pellicle, but grows in the form of clumps along the test-tube wall, or as a flocculent deposit at the bottom of the test-tube. In glucose broth the growth is granular but not so abundant as that of the diphtheria bacillus. It forms acid in 24 hours, but not so strongly as the diphtheria bacillus. It forms no visible growth on potato.

Pathogenicity. The diphtheroid bacillus has a feeble pathogenic action on young rats. Three out of four young rats that received 2 c.c. of a broth culture a week old died within a week. Old rats proved resistant.

Agglutination tests.

No great importance can be attached to these agglutination tests in view of the absence of specificity which some observers have demonstrated in regard to the agglutination of the acid-fast group of bacilli. The tests are of a preliminary character and are included in this paper merely as an indication of a line of research which it was thought might throw some light on the relationship of the organisms.

Preparation of bacterial suspension. A piece of tissue, very rich in the acid-fast bacillus of the rat, was removed from the methylated spirit in which it had been preserved, allowed to dry, and ground up in a mortar with glass powder in normal salt solution. After standing for some time it was found that nearly all the fine pieces of tissue had sedimented, and that the supernatant opalescent fluid consisted almost entirely of acid-fast bacilli. The fluid was brought to a degree of opacity suitable for carrying out naked eye tests in Wright's agglutination tubes which were used on account of a very small quantity of serum being available. The bacterial suspension remained almost unaltered for 24 hours.

TABLE I. *Result in three hours.*

TEST 1.

Dilution	Normal Serum	Leprous Serum
1 : 2	Nil	Large sediment
1 : 4	,,	,, ,,
1 : 8	,,	,, ,,
1 : 16	,,	,, ,,
1 : 32	,,	Distinct sediment
1 : 64	,,	Trace

TABLE II. *Result in sixteen hours.*

TEST 2.

Fresh Dilutions	Normal Serum	Leprous Serum
1 : 2	Nil	Large sediment
1 : 10	,,	,, ,,
1 : 20	,,	,, ,,
1 : 40	,,	Distinct sediment
1 : 50	,,	Trace
1 : 60	,,	Nil

TABLE III. *Agglutination of diphtheroid bacillus emulsion prepared from agar culture of three days' growth by means of shaking with glass beads.*

Serum	1 : 2	1 : 10	1 : 20	1 : 30	1 : 40
Normal (human)	0	0	0	0	0
Human (tubercular case)	0	0	0	0	0
Lepra serum	+	+	+	+	0
Normal rat	0	0	0	0	0
Rat (inoculated with acid-fast bacilli)	+	+	0	0	0
Rat 2 (ditto)	+	+	0	0	0

In these tests:—

(1) the serum from a case of human leprosy agglutinated the acid-fast bacillus from the rat; normal human serum had no agglutinating power;

(2) the serum of rats inoculated with the acid-fast bacilli agglutinated the diphtheroid bacillus; normal rat's serum did not agglutinate the bacillus;

(3) normal human serum and the serum from a tuberculous patient failed to agglutinate the diphtheroid bacillus, whereas the serum from a case of leprosy had distinct agglutinating properties.

Note. The serum from only one normal, one tuberculous, and one leprous patient, was used in these tests. I wish here to express my thanks to Dr Lie of Bergen for his kindness in supplying some blood from a case of leprosy.

Inoculation Experiments.

I. Rats.

Thirty black and white rats have been inoculated with material consisting of emulsions made from the infiltrated skin and enlarged lymphatic glands obtained from the naturally, or experimentally, infected animals. A considerable number of these died of intercurrent disease without obvious signs of having been infected with the acid-fast bacillus, though in many of them, even in the absence of naked-eye changes, the organism could be demonstrated to be present in the peritoneum after two or three months. Nine animals, however, showed marked lesions, evidently produced by a multiplication and invasion of the acid-fast bacillus, and in some of these the lesions were of a very striking character.

In three cases the infection was carried on successfully from the naturally infected animal to an experimental animal and from the experimentally inoculated rat to a third rat: the lesions in the two generations of experimentally infected animals were similar.

Course of the Disease in the Inoculated Rats.

The disease runs a very slow course in the inoculated animals. In one case a rat died about one year after inoculation with well-marked evidence of a progressive invasion of the bacillus. In others well-marked lesions developed and death occurred within six months of inoculation. There was no definite evidence of increase of virulence in the third generation.

Pathology.

Subcutaneous Inoculation was in several instances followed by the production of a local lesion but this was not a constant result. The lesion consisted of a nodule containing a semi-caseous-like substance resembling the material which was present in the skin infiltration of the naturally infected animals. The acid-fast bacilli were very numerous in these local lesions. The lymphatic glands in such cases, even at a distance from the lesion, were sometimes invaded by the bacilli and showed the changes already described. For example, in one case where the local lesion was situated in the groin the axillary lymphatic glands were infected.

Intraperitoneal Inoculation. The most marked lesions resulted from intraperitoneal injections. In these the epiploön presented a

striking appearance. It was frequently infiltrated with grey opaque looking masses of material which on microscopical examination proved to be masses of cells containing bacilli. The peritoneum was in some cases thickened, and pale yellow patches of infiltration occurred on both the visceral and parietal surfaces.

Liver. As a rule, even when the infection was well-marked, the liver showed nothing abnormal or the capsule only was involved. In one case, however, the organ was beset with small, pale, yellow, sharply circumscribed areas to such an extent that about a third of the liver substance seemed to be destroyed. A second case showed the condition in a less advanced stage. The areas under the microscope were seen to be sharply circumscribed and surrounded by flattened liver cells. The process was very extensive close to the peritoneum and seemed to extend along the Glisson's capsule. When stained by Ziehl-Neelsen's method the appearance presented under a low power is shown in Plate VII, Fig. 1, which is typical of what is found throughout the liver. The bulk of each nodule appears to be composed of masses of acid-fast bacilli. At the periphery these are mostly arranged in round masses as if lying in cells or cell remains. Some of these round masses are very large and so tightly packed with bacilli that they appear almost homogeneous. In the centre of the nodule the cellular appearance may be less marked, many of the bacilli appearing to be free. Round some of the nodules the capillaries are greatly dilated, and in the endothelium of these there are groups of acid-fast bacilli.

Spleen. The capsule was thickened and contained bacilli in several cases, but only in one was the organ itself markedly involved. This occurred in the case in which the liver was most affected.

Kidneys, Bladder, and Genital Organs. In several instances the capsules of these organs were thickened and infiltrated with bacilli but so far as could be found no extension of the process to the organ itself had occurred. In the case of the testicle and epididymis groups of bacilli were found lying in the connective tissue framework of the organ.

Thorax. In three of the inoculated animals a very striking appearance was found on opening the thorax. A tumour-like mass occupied the anterior mediastinum, and the posterior surface of the sternum was covered with an infiltrating material which in one case reached a thickness of a quarter of an inch. The pale yellow tumour-like mass occupied a considerable part of the thorax and was moulded over the pericardium and apices of the lungs. On microscopical

examination only small areas of lymphatic gland tissue were visible, the bulk of the mass consisting of the acid-fast bacilli. This tendency, especially in rodents, for the anterior mediastinal glands to become infected from the peritoneum has been frequently observed.

Lungs. Lesions of these organs were not common, but in two cases a few small nodules, the size of millet seed, were observed. These appeared to originate from the lymphatic vessels lying beside the small bronchi and vessels.

The *Pericardium* and *Epicardium* were secondarily invaded in these cases with retrosternal tumours.

Myocardium. A small nodule containing the bacilli was in one case found in the myocardium.

Blood. On several occasions bacilli were seen lying within the blood-vessels.

The Central Nervous System. In two cases the bacilli were looked for in the brain and spinal cord but were not found.

Nerve-trunks. Bacilli were found to have invaded these both in the experimental animals and in those naturally infected.

II. Inoculation of Animals other than Rats.

Guinea-pigs. Thirteen animals were inoculated; of these only one developed a small local subcutaneous lesion which contained the acid-fast bacilli. None of the others showed any effect.

Rabbits. Six animals inoculated; showed no effect.

Mice. Ten animals inoculated. Six of these died, but without showing any lesions which could be attributed to the acid-fast bacillus.

Monkey (*Macacus rhesus*). After 18 months showed neither local nor general signs of infection.

These results show that we are not dealing with Tubercle bacilli, especially as in some of the guinea-pigs and rabbits very large doses were given.

Remarks and Conclusions.

One of the chief features common to this disease in the rat and to lepra in the human subject, namely the presence of the bacilli in such large numbers within the cells, has been regarded by Babes as the evidence of a form of symbiosis between the cells and the bacilli. The bacilli invade the cells and live within them without interfering with their vital functions.

It seems probable, however, that we are both here and in leprosy dealing with the phagocytosis of a relentlessly invading germ of great resistance, which, notwithstanding the fact that it finds itself in a suitable medium, has little power of producing toxic substances. In the case of tubercle, after a few bacilli have been taken into a cell, so much toxin is secreted by the bacilli that necrosis and death of the cell occurs. Here, on the other hand, the bacilli when taken into the cells are able to continue their growth and having only feeble toxigenic properties they have relatively little deleterious action on the cells, which maintain their vitality and for long continue to struggle against the invaders. The slowness of the struggle may possibly account for the enormous size of the giant cells.

It would be out of place here to go into the literature of the attempts and failures to inoculate animals with the Lepra bacillus; one may, however, refer to the work of Iwanow (1902), who, working in Metchnikoff's laboratory, succeeded in obtaining results in guinea-pigs which he regarded as indicating that a certain multiplication of bacilli may have taken place. The appearances which he observed in the epiploön of the guinea-pig resemble those observed in the slighter form of invasion in the case of the rat disease. It would be of interest to know whether the rat has been used for inoculation experiments with leprous material. So far as could be learned from a brief review of the subject this does not appear to have been the case [Wolters (1893); Finger (1901), and Hansen (1902)].

The features of resemblance between these two diseases are so strong that they point to a close relation between the associated bacilli.

The consideration that man and the rat are fellow victims of almost equal susceptibility to other disease germs such as the plague bacillus, might even suggest the possibility that in lepra and in this rat disease we are dealing with the same micro-organism affecting two species.

There is no intention here, however, in the present state of our knowledge to uphold such a hypothesis, which is obviously rendered improbable from epidemiological considerations alone.

As has been indicated in discussing the interpretation of the appearance in the infected animals, many of the pathological features of resemblance probably depend on the slow invasion of, and the defence offered by, the animal's tissues against a highly resistant organism which is not markedly toxigenic.

CONCLUSIONS.

1. This disease of the rat has a remarkable resemblance, both in its pathological, anatomical, and in its bacteriological features to Lepra in the human subject.

2. All attempts at cultivation of the acid-fast bacillus have failed.

3. A diphtheroid bacillus has been isolated and cultivated from two cases of the disease, but so far attempts to convert this into an acid-fast form have been unsuccessful.

4. The experiments on agglutination having been of a preliminary character do not permit as yet of any definite conclusions. Nevertheless the bacillus was agglutinated by the serum of a leprous patient.

5. The disease could be conveyed to other rats (but not to rabbits, guinea-pigs, mice or a monkey) by subcutaneous and by intraperitoneal injection of emulsions of the bacilli from infected tissues.

6. A large number of the rats inoculated succumb to intercurrent diseases but a certain number develop well-marked characteristic lesions which can be again reproduced by reinoculation.

7. The disease in these artificially infected animals runs a very slow course, which may extend to about one year.

EXPLANATION OF PLATES VI AND VII.

Plate VI. Fig. 1. Photograph of rat (*Mus decumanus*) showing the appearances presented in a case of natural infection with the acid-fast bacillus. Note the patches of alopecia, two of which show small ulcers.

Fig. 2. Photograph showing a dissection of the enlarged cervical and axillary lymphatic glands in a case of natural infection of the rat with the acid-fast bacillus.

Plate VII. Fig. 1. Drawing of the microscopical appearances presented by a section of the liver of a white rat which had been inoculated intraperitoneally six months before death. The red colour of the affected areas in the liver is due to the presence of masses of the acid-fast bacillus. Ziehl-Neelsen's solution, 3 $^0/_0$ hydrochloric acid-alcohol, aqueous methylene blue. $\times 100$.

Fig. 2. Drawing of a giant cell containing bacilli. From a smear preparation of the axillary gland of a white rat which died eleven months after subcutaneous inoculation. Ziehl-Neelsen's solution, 25 $^0/_0$ sulphuric acid, aqueous methylene blue. Under oil immersion lens. $\times 825$.

Fig. 3. Drawing of the microscopical appearances of a section of an infected lymphatic gland. Shows the cells of endothelial origin packed with acid-fast bacilli. Ziehl-Neelsen's solution, 3 $^0/_0$ hydrochloric acid-alcohol, aqueous methylene blue. $\times 825$.

Fig. 4. Same cell as seen in Fig. 2. $\times 100$.

Fig. 5. Drawing of the microscopical appearances of a section through the affected skin of a rat suffering from the naturally acquired disease due to the acid-fast bacillus. Most of the red colour below the epithelium is due to the presence of large numbers of bacilli. Stained with Ziehl-Neelsen's solution, 25 $^0/_0$ sulphuric acid, aqueous methylene blue. $\times 100$.

The drawings are by Mr M. H. Lapidge.

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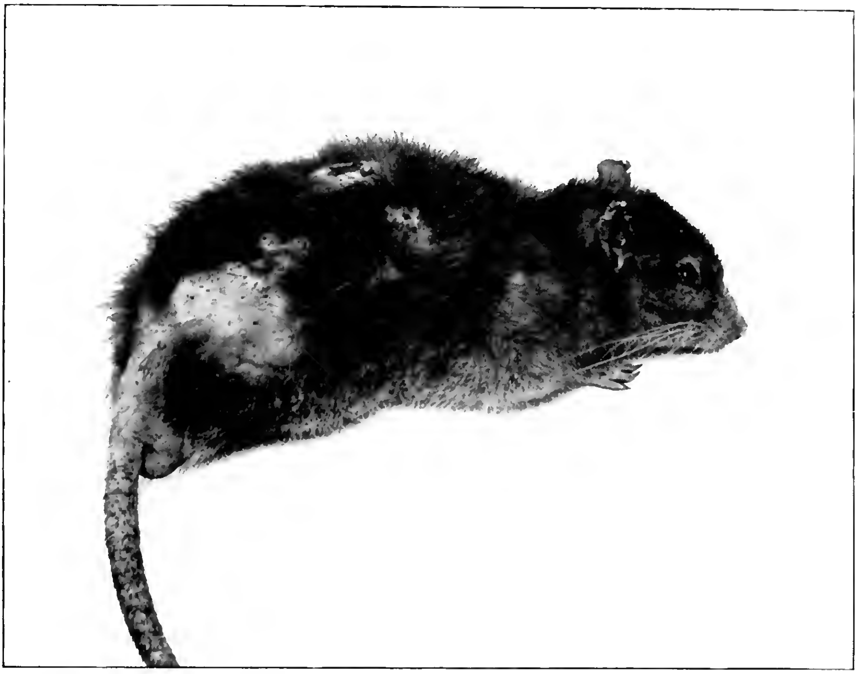
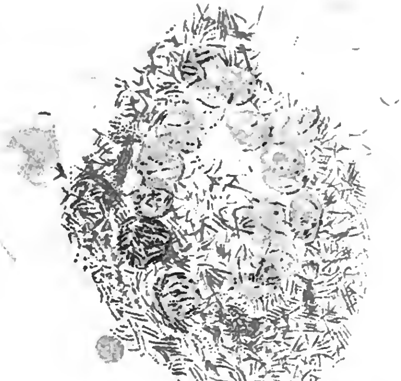
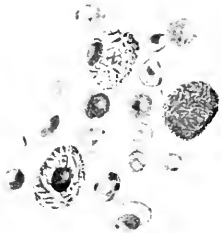
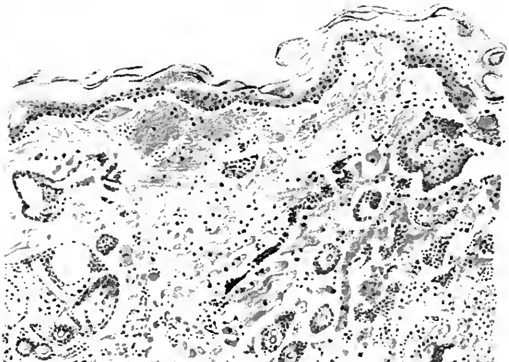


Fig. 1.

Fig. 2.







ON THE MECHANISM OF AGGLUTINATION.

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In view of the large number of hypotheses advanced to account for the phenomenon of agglutination of bacterial cells, it was suggested to me by Dr Allan Macfadyen, that I should investigate the agglutination of typhoid bacilli from a physico-chemical standpoint. I here beg to express my indebtedness to Dr Allan Macfadyen and Dr George Dean for much kindly aid and criticism.

In a preliminary notice to the Pathological Society (13. II. 1904), I indicated that my results supported the view first advanced by Bordet, that the phenomenon of agglutination may be divided into two stages:

1. Fixation of agglutinin by bacilli.
2. Aggregating influence of salts and other substances.

Further, I suggested that the fixation obeyed the general laws of staining and that the aggregation was a surface-tension effect. In this investigation the first question was whether agglutination was a strictly specific phènomenon or one of a general character. Agglutination is not entirely specific. According to Köhler (1900) typhoid bacilli are agglutinated not only by typhoid immune serum but also to a smaller extent by the sera obtained from *B. coli,* pneumococcus and meningococcus infections. Lubowski and Steinberg (1904) obtained agglutination by immune sera from proteus bacilli and staphylococcus, and Rodet (1904) found that the normal serum of the rabbit, etc. if not too much diluted agglutinated typhoid bacilli. Stern (1903) has shown that the serum produced by a bacillus generally agglutinates allied bacilli but is specific in the sense that the bacillus itself is most easily agglutinated. In addition to the specific agglutination caused by immune sera, a non-specific agglutination can, according to Malvoz (1897), be brought about by saffranin and other dyes, and Gilardoni (1903) states that, in general, any reagent which precipitates proteids will cause agglutination.

Since the most diverse fluids thus lead to the same end the reaction cannot be regarded as a definite chemical change, and further investigation is required to ascertain whether the physical part of the reaction (or mechanism) is the same in all cases.

The chief views regarding the essential nature of agglutination which have been put forward are those of Pfeiffer, Gruber, Paltauf, and Bordet.

(1) Pfeiffer (1896) and Emmerich and Löw (1899) regarded agglutination as a vital paralysis of the bacilli due to the action of a bacteriolytic enzyme. It has, however, been shown by Joos (1901) that the agglutinating substance in typhoid immune serum is in the absence of salts absorbed without diminishing the motility of the bacilli, and further, that after the agglutination is completed by the addition of an electrolyte, *e.g.* sodium chloride, reproduction does not cease. Moreover, the enzymic character of the agglutinating substance, or agglutinin, is rendered doubtful by the fact that the bacteriolytic enzymes are destroyed by temperatures at which the agglutinins remain unaffected.

(2) Gruber (1899), Dineur (1898), and Nicolle (1898), supposed that a glutinous substance, "glabrificin," was absorbed from the serum by the bacilli, thereby causing small adhesive prominences on the membranes, or rendering the flagella, or the membranes themselves, adhesive on contact.

These hypotheses, which ascribe agglutination to the adherence of bacilli after contact, are insufficient, however, to account for the movement of dispersed non-motile bacilli towards a common centre.

(3) Paltauf (1897) and Duclaux considered that a specific precipitate is produced in the medium which during flocculation mechanically carries the bacilli with it.

Wassermann (1903) and Kirstein (1904) have shown that the precipitable substance in a culture filtrate and the agglutinable substance in the bacilli are very closely related, and that the former may be regarded as agglutinable substance ejected by the bacilli into the medium.

Beljaeff (1904), however, finds that the agglutinating power of a typhoid immune serum is not parallel to its power of precipitating a typhoid filtrate, and Norris (30. v. 1904) considers that many facts point to at least some of the substances concerned in specific agglutination and specific precipitation being distinct.

That agglutination can be brought about by a visible precipitate

is certain, and that extremely fine precipitates are sometimes formed round the isolated organisms during agglutination, has been shown by Löwit (1904), but agglutination of *B. typhosus* has been observed by Hinterberger where no precipitate was demonstrable, and it remains an open question whether precipitation in the medium is a necessary part of the mechanism of agglutination. As regards precipitation within the bacilli it will be seen from the following (part 2) that this is highly probable.

(4) Bordet (1899) separated the mechanism of agglutination into two stages as mentioned above, (1) fixation, and (2) aggregation. The fixation of agglutinin by the bacilli he considers to be analogous to the fixation of a dye by a tissue, and that once the agglutinin is fixed, the bacilli obey the laws of inert particles, and that aggregation is caused by changes in the molecular attraction between the organisms and the surrounding medium.

Since staining is usually regarded as a special case of adsorption, *i.e.* the condensation or concentration of gases or dissolved matter on substances with highly developed surfaces, one might expect that the fixation of agglutinin would obey the general laws of adsorption. If the second part of the hypothesis is correct, since the relations between inert particles and a surrounding medium are directly governed by the surface tension of the particles against the medium, one would expect the bacilli to be subject to corresponding surface tension effects.

It being, therefore, possible to control both parts of Bordet's view by direct experiments on adsorption and surface tension, an investigation on these lines was instituted.

The Fixation of Agglutinin.

According to Arrhenius (1903) agglutinin is partitioned between bacilli and medium much as iodine is between water and carbon-disulphide. He further deduces that the molecular weight of agglutinin dissolved in the medium is one and a half times that of the agglutinin dissolved in the bacilli.

The following objections may be raised to the views advanced by Arrhenius. Specific agglutinins and the agglutinable substances, precipitins and precipitable substances, toxins and antitoxins, belong to the same class of substances. (Bordet, 1903.) This class also includes lysins and antilysins, immune bodies and complements with their antibodies. All these substances belong to the division of

colloidal solutions of compounds of high molecular weight. (Müller, 1903.)

Toxins, lysins, and agglutinins are possessed of some degree of diffusibility, but in general the substances with which they react have a very low rate of diffusion of the order of magnitude of that of globulin and other typical colloids. (Billitzer, 1903.) A recent determination of the molecular weight of a well-purified typical organic colloid, viz., Glycogen, by the Nernst-Abegg cryoscopic method according to Gatin-Gruzewska (1904), gave no depression of the freezing-point of the aqueous medium. Glycogen is then either a difficultly soluble substance with a molecular weight over 140,000, or it is incapable of forming a true solution and its molecular weight *indefinite.* This glycogen migrated in an electric potential gradient like other colloids, to the anode. Sabanejev had previously found for glycogen the molecular weight 1620, probably owing to the presence of crystalloidal impurities.

E. W. Reid (1904) using Starling's (1899) method found that by washing salted out or crystallised proteids, solutions of proteids are obtained which give no osmotic pressure against a membrane of gelatine.

The view that colloidal solutions of high molecular organic compounds are not in homogeneous solution is supported by their dispersion of polarised light. Raehlmann (1904), Römer and Siebert (1904), using the Siedentopf-Zsigmondsky microscope, have observed in serum and other albumin, globulin, and glycogen solutions a similar granular appearance to that exhibited by colloidal gold, silver, and platinum. Taken in conjunction with the low diffusibility and absence of osmotic pressure this granular structure seems to indicate that typical colloidal proteids are almost entirely in suspension, and that the pseudo-solutions are really heterogeneous systems. The conditions under which the laws of Boyle, Gay-Lussac, and Avogadro apply being absent, it is probable that for proteids of this class the application of the gas laws in the manner made by Arrhenius is not a true interpretation of the mechanism of their reactions and equilibria with other substances. Nernst (1904) has recently pointed out that for reactions in heterogeneous systems, in so far as they take place entirely or partly on the interfaces of the different phases, the velocity of reaction depends entirely or partly on velocities of diffusion, which in general have nothing to do with the order of a reaction, that is, with the number of reacting molecules. Since at least one of the substances in the reactions with which we are concerned must be considered as in the typical colloidal state

van't Hoff's laws cannot be applied to the reactions of specific substances with their antibodies.

On the other hand, typical colloidal solutions being heterogeneous systems with highly developed interfaces, the velocities of their reactions with other substances, and the equilibria they attain, should be governed by the laws which hold for the absorption of substances from solutions by suspensions, porous substances, and highly developed surfaces in general, classed generally as adsorption phenomena.

That there are quantitative laws of adsorption has been fully recognized by Ostwald (1891). A dilute solution of hydrochloric acid digested with animal charcoal slowly reaches equilibrium, which does not change when portions of the solution or of the charcoal are removed. The equilibrium is then only dependent upon the ratio of the concentration of hydrochloric acid in the solution to that in the charcoal. On the addition of water the charcoal gives up a portion of its hydrochloric acid within a few minutes and a new equilibrium is established. A given mass of charcoal can be obtained in this manner so uniform in concentration as regards hydrochloric acid that the experiments agree within fractions of a percentage. With various substances, the amounts absorbed by charcoal, when plotted as ordinates against the amounts of water as abscissae in right-angled coordinates, gave hyperbolic curves. The laws governing the absorption of the most varied substances are the same, yet, in many cases the charcoal undoubtedly reacts chemically on the substance absorbed. It is important to observe that the state of equilibrium is easily and rapidly attained when the solid body is treated with a concentrated solution of the substance to be absorbed and subsequently by addition of water brought to the desired dilution. On the contrary, if the solid substance be brought into contact with the already diluted solution days may elapse before equilibrium is attained.

A further important feature of adsorption equilibria is that the general formula

$$C_1 = KC_2{}^n$$

is capable of wide application, where C_1 is the concentration of the free substance in the aqueous medium, C_2 the concentration of the bound substance in the adsorbing material, K and n constants dependent on the chemical nature of the substances brought into contact. Thus when a solution of iodine is mixed with animal charcoal the relation is

$$C_1 = 0{\cdot}0014\ C_2^{4}.$$

Schmidt (1904) found that acetic, succinic, and oxalic acids with charcoal, similarly give definite and characteristic constants K and n. It may be well to observe that the values of n and K do not give any definite idea of the magnitude of the adsorbed molecule or grain as compared with that in the aqueous medium but rather of the relative intensity and capacity of adsorption of charcoal and water for the substances partitioned.

It has been observed that colloids are adsorbed by charcoal, which leads to the consideration of the more immediately interesting case in which an inferior colloid or a crystalloid is adsorbed by a typical colloid.

For this we can assume within certain limiting concentrations that

$$C_1 = KC_2^n$$

where C_1 is the concentration of inferior colloid that is retained in the aqueous medium as a consequence of the solvent-like attractive or adhesive intensity of the latter, C_2 is the concentration of inferior colloid that is bound or taken up by the typical colloidal medium in virtue of its adhesive intensity.

From Eisenberg and Volk's (1902) experimental investigation of the equilibria which obtain between agglutinin and typhoid bacilli, Arrhenius has deduced the relation

$$\frac{C}{A} = KB^{\frac{2}{3}}$$

where $\frac{C}{A}$ represents the concentration of agglutinin in the bacilli, B the concentration of free agglutinin in the medium, and K is a constant. This equation is, it seems to me, a special case of the adsorption formula where the power $\frac{2}{3}$ has only significance in indicating the relative intensity of adsorptive power of the medium and the bacilli for agglutinin. The validity of the special formula given is however questionable, for in calculating the agglutinating value of a serum an assumption has been made by Eisenberg and Volk which is very open to criticism. They take it for granted that a serum which gives a concentration of agglutinin per c.c. equal to one 24 hours' unit when diluted to 20,000 times its original volume, will contain in 1 c.c. of the original serum 20,000 units of agglutinin. In other words, they assume that the number of agglutinin units present in a fluid is independent of its volume, no association, dissociation of the units, or, in other words, no change in the binding capacity of the agglutinin units takes place. When we consider that flocculation by sera and other chemicals reaches

a maximum at certain concentrations, *i.e.* that there are optimum concentrations of sera, etc., above and below which the flocculation is diminished, it seems to me, that without further experimental investigation, we cannot assume that the fixation of agglutinin is unaffected by this phenomenon or that the binding capacity of the diluted unit is equal to that of the same quantity of agglutinin when in the concentrated state. The experimental error being the same for the estimation of free agglutinin in dilute and concentrated solutions, the deviations from the calculated values for dilute solutions shown by Eisenberg and Volk's figures prove that Arrhenius's formula, as it stands, does not embrace the entire range of dilutions. In Table III (Arrhenius, 1903, p. 417), assuming that in more dilute solutions we have a different relation, we obtain a better constant by using the formula

$$\frac{C}{A} = KB^{\frac{3}{4}} \text{ for the more concentrated solutions, viz.,}$$

$$K = 13{\cdot}6,\ 12{\cdot}3,\ 12{\cdot}5,\ 12{\cdot}54,$$

whereas

$$\frac{C}{A} = KB^{\frac{2}{3}} \text{ gives } K = 23{\cdot}2,\ 24{\cdot}8,\ 26{\cdot}3,\ 28{\cdot}2.$$

Again, rejecting according to the principle adopted by Arrhenius the last value of Table XIII we obtain for $\frac{C}{A} = KB^{\frac{1}{2}}$, $K = 23, 17, 23{\cdot}2, 24{\cdot}5$, which seem to be as good as $\frac{C}{A} = KB^{\frac{2}{3}}$, $K = 12{\cdot}7,\ 11{\cdot}8,\ 8{\cdot}4,\ 10{\cdot}6$. But a change in the power to which B is raised would give from Arrhenius's standpoint a radical change in the molecular relations of agglutinin, for then the molecular weight of free agglutinin would be to that of bound agglutinin as 4 is to 3 or 2 to 1, entirely different dissociations to that found in other cases. From the adsorption point of view the variation of K and n from one serum to another is to be expected. From the hypothetical gas-law standpoint, however, K and above all n must be constant. Moreover, as Arrhenius himself points out, if multiples of a certain concentration of agglutinable substance and agglutinin be brought together the power $\frac{2}{3}$ is incompatible with Eisenberg and Volk's experimental series in which they find the ratio between agglutinin bound and free to be unchanged on bringing multiple concentrations together. It seems to me that this last result of Eisenberg and Volk would be explicable on the partition formula $\frac{C}{A} = KB$, and this would lead to the conclusion that n was dependent

upon the conditions of the experiment. The apparently constant active mass of the agglutinable substance within the bacilli cannot be ascribed to the non-participation of the cell contents in the reaction, for, as we have seen, Wassermann (1903) has demonstrated that agglutinin reacts with precipitable substance and gives rise to a precipitum, and we have no reason to assume that this reaction does not occur within the bacilli when agglutinin is absorbed; on the contrary, the second phase of agglutination is most easily accounted for by the assumption that the cell contents experience a decided change in structure, either physical or chemical, on the absorption of agglutinin. From a physico-chemical point of view it does not then seem probable theoretically that the gas laws apply to the fixation of agglutinin by agglutinable substance, nor experimentally that there is foundation for the application. As far as the equilibria are concerned, a superficial analogy is, however, to be expected, such as the phenomena of adsorption would lead us to expect. With respect to the more general problem of the velocity of reaction of substances which may be classed with agglutinin, and agglutinable substance such as toxin and antitoxin, the agreement between the experimentally observed relations and those calculated by Arrhenius is also merely superficial. Similar agreement between observations on the velocity of reaction in heterogeneous systems and calculations on the assumption of homogeneity has been observed by Hantsch (1904), who recognised fully the untenability of the assumption, for the absorption of ammonia gas by solid organic acids and of hydrogen chloride by solid amine bases.

With a view to the elucidation of the class of phenomena to which the fixation of agglutinin may be attributed the following experiments were carried out:

The unit concentration of agglutinin was arbitrarily fixed as that dilution of a serum 1 c.c. of which on addition to 1 c.c. of a standard suspension of typhoid bacilli in a tube 6 mm. in diameter showed clumps just visible with the aid of a hand-lens at the end of $2\frac{1}{2}$ hours, at room temperature. The same lens was used throughout the series compared. In thus reducing the time at which the reading took place I hoped to eliminate part of the error due to the growth of a living culture in a very weakly agglutinating fluid. In Eisenberg and Volk's experiments this error may have influenced their results, for they allowed their test mixtures to remain 24 hours before reading. The absolute quantity of agglutinin represented by a unit depends upon the time given to the test mixture to flocculate, a $2\frac{1}{2}$ hours' unit contains more agglutinin

than a 24 hours' unit, hence when an agglutinating value is ascribed to a fluid the time value of the unit must be given.

The standard suspension was prepared from an agar culture of a good agglutinating strain, grown at 37° C. for 18 hours. Each agar tube was rinsed out with 15 c.c. of 0·82 % sodium chloride. To secure uniformity the suspensions, obtained from a dozen tubes, were mixed and filtered through glass-wool to remove agar particles.

The typhoid immune serum first employed was obtained from Horse No. 1 on December 5th, 1903, and gave agglutination at a dilution of 1 in 40,000, equivalent to an agglutinating value of 40,000 diluted $2\frac{1}{2}$ hour units.

Since the use of killed cultures might reduce the experimental error, a few comparative series, to test the relative agglutinability of the above suspension and of the standard suspension after it had been heated to 58° C., for 30 minutes, were carried out and gave as a mean the following result. With the unheated standard suspension :—

4 Series.

Serum concentration	1/20	1/200	1/2,000	1/20,000	1/40,000
Time of agglutination	8	19	29	40	150 minutes

With the heated standard suspension :—

4 Series.

Serum concentration	1/2,000	1/8,000	1/20,000
Time of agglutination	10	80	150 minutes

The serum in the higher dilutions, corresponding to the $2\frac{1}{2}$ hour units, showing from this only half the agglutinating power on the heated bacilli of that which it has for the unheated, I decided to use the unheated bacilli, as they form a more delicate indicator of diluted free agglutinin in a solution. Again, since heating probably alters the nature of the absorbing substance and since from the above it seems probable that the laws which hold for the fixation of agglutinin by the heated agglutinable substance would be different to those governing the fixation in the unheated substance, the unheated standard suspension was also used for the experiments on equilibrium. A further investigation to ascertain whether the motility of the bacilli was connected with their agglutinability gave entirely negative results. Two non-motile cultures obtained by 18 transfers on agar at 42° C., gave values for their agglutinability which were, within the experimental error, identical with those given above for the unheated standard suspension. On staining

by Loeffler's method the flagella were observed in this non-motile culture to be still attached to the cell bodies. That the contents of the cell play an important, if not the chief, part in the fixation of the agglutinin seems to be indicated by the fact that one sample of typhoid residue obtained by grinding the organisms at the temperature of liquid air and washing repeatedly with distilled water only gave agglutination with the above immune serum in the concentration of 1/20; a second sample did not agglutinate even with the undiluted serum. This result is in entire harmony with Bordet's view.

With regard to the minutiae of the experimental method it is important that after mixing bacilli and agglutinating fluid the mixture be not disturbed. To trace the possible effect of shaking, the heated culture, which with serum in a concentration of 1 in 2,000 agglutinated in 10 minutes, was shaken up for 1 minute, the bacilli reagglutinated in 1 hour; on repeating the agitation a third agglutination took place in 3 hours. This result is also interesting in showing that the second phase of agglutination, *i.e.*, the aggregation, is quite distinct from the fixation of agglutinin which is completed in a very short time.

The equilibria existing between various concentrations of serum and a constant concentration of bacilli were then determined:—10 c.c. of various dilutions of serum were added to equal volumes of suspension, thoroughly mixed and allowed to stand 3 hours at 17° C., the whole was then centrifugalised, the supernatant fluid decanted and the dilution in which it just caused agglutination in 2½ hours determined. The results obtained expressed in the manner adopted by Eisenberg and Volk and the value of K calculated by Arrhenius's formula were as follows:—

Serum of Horse No. 1 = 40,000 2½ *hour units.*

Mean values obtained from 6 Series.

Concentration of serum	Units of agglutinin added	Units of agglutinin absorbed	Units of agglutinin free	K
1/20	2,000	1,300	700	16·5
1/30	1,333	1,133	200	38·9
1/40	1,000	840	160	28·5
1/50	800	768	32	76·2
1/60	666	646	20	87·6
1/200	200	200	0	—

With the exception of the 1/30 dilution the value of K calculated according to the formula of Arrhenius shows a constant increase.

Taking Eisenberg and Volk's (1902) agglutinating unit, viz.—the dilution of agglutinating fluid 1 c.c. of which on addition to 1 c.c. of

the above-mentioned bacterial suspension showed a definite sediment after 24 hours—and using another serum, less regular results were obtained. 15 c.c. of suspensions were mixed with 15 c.c. of serum,

$$S, \frac{S}{2}, \frac{S}{4}, \frac{S}{8}, \frac{S}{16}, \frac{S}{32}, \frac{S}{64}, \frac{S}{128}, \frac{S}{256}, \text{ and } \frac{S}{512}.$$

The highest concentration of serum was obtained by adding 30 c.c. serum to one agar tube of culture. The values obtained were as follows:—

Serum of Horse "Tom" = 44,000 24 hour units.

Mean values obtained from 4 Series.

Concentration of serum	Units of agglutinin added	Units of agglutinin bound	Units of agglutinin free
1/1	44,000	24,000	20,000
1/2	22,000	8,000	14,000
1/4	11,000	3,000	8,000
1/8	5,500	500	5,000
1/16	2,750	750	2,000
1/32	1,375	675	700
1/64	688	488	200
1/128	344	329	15
1/256	172	170	2
1/512	86	86	0
1/1,024	43	43	0

The mixtures were heated for 2 hours at 37° C., and then allowed to stand 24 hours. No constant could be found by Arrhenius's formula for these figures.

Owing to the wide deviations obtained from Arrhenius's formula the question arose whether equilibrium between bound and free agglutinin could be easily and certainly attained or whether we had here only an apparent or so-called "false" equilibrium. The serum of the horse "Tom" (S) was brought together with a suspension of typhoid bacilli (T) of a concentration equal to one agar tube of culture grown for 18 hours at 37° C., in 5 c.c. of saline. The concentrations $\frac{S}{3}$ and $\frac{T}{3}$ were obtained by a dilution with saline. The final volume was 30 c.c., and the final concentration of serum and bacilli in all cases was the same. The following mixtures were made:—

2 Series.

I. 15 c.c. $\frac{S}{3}$ + 15 c.c. $\frac{T}{3}$.

II. 15 c.c. $\frac{S}{3}$ + 5 c.c. $\frac{T}{3}$, after 18 hours + 10 c.c. $\frac{T}{3}$.

III. 15 c.c. $\frac{S}{3}$ + 5 c.c. T, after 18 hours + 10 c.c. saline.

IV. 15 c.c. $\frac{T}{3}$ + 5 c.c. $\frac{S}{3}$, after 18 hours + 10 c.c. $\frac{S}{3}$.

V. 15 c.c. $\frac{T}{3}$ + 5 c.c. S, after 18 hours + 10 c.c. saline.

VI. 5 c.c. T + 5 c.c. S, after 18 hours + 20 c.c. saline.

VII. 5 c.c. T + 5 c.c. S, after 2 hours + 20 c.c. saline.

VIII. 15 c.c. $\frac{T}{3}$ + 15 c.c. $\frac{S}{3}$, mixed at the time of addition of the second portion in the other cases.

After allowing to stand for 24 hours further, the supernatant fluids had practically the same agglutinating values, the variations being at the most 200 units in 8,000 free units of agglutinin measured by bringing 1 c.c. of the fluid together with 1 c.c. of $\frac{T}{3}$ and heating to 37° C., for 2 hours.

Given a considerable time then we attain a fairly constant equilibrium with a concentrated serum. This equilibrium was found, however, to show the characteristics of "false" equilibria, when the serum was more diluted. *The successive addition of the bacillary suspension to the serum removed more agglutinin from the solution than when the addition was made at one time.* 10 c.c. of the diluted serum of Horse No. 1 were added to 10 c.c. of the standard suspension at once, and in a second case at the rate of 5 drops every 5 minutes. The concentrations of serum used were $\frac{S}{10}$ and $\frac{S}{200}$. The equilibria seemed to be, within the experimental error, uninfluenced by the rate of addition. When, however, 10 c.c. of the suspension were gradually added to an equal volume of the $\frac{S}{10}$ concentration of serum, the free agglutinin corresponded to 95 units ($2\frac{1}{2}$ hr.) when the addition was made at one time, whereas for the addition in parts 60 units ($2\frac{1}{2}$ hr.) were left free.

The serum of the horse "Tom" of concentration $\frac{S}{10}$ was then mixed with the standard suspension in the following manner:—

2 Series.

I. 15 c.c. $\frac{S}{10}$ + 15 c.c. suspension, centrifugalised after 3 hours.

II. 15 c.c. $\frac{S}{10}$ + gradually increasing fractions of suspension.

III. 15 c.c. suspension + gradually increasing fractions of $\frac{S}{10}$.

IV. 15 c.c. $\frac{S}{10}$ + 15 c.c. suspension, centrifugalised after 30 minutes.

In II and III the entire addition of the second 15 c.c. was spread over $2\frac{1}{2}$ hours and the mixtures centrifugalised after a further 30 minutes. The supernatant fluids of I, III and IV showed 2,600 units of free agglutinin in a 2 hours' test at 37° C., whereas II showed 2,400 units; I, III and IV gave 3,200 six-hour units and II 2,800. Since I and IV gave the same result the agglutinin equilibrium is set up in less than 30 minutes at room temperature. No. II confirms the view that we are dealing with a "false" equilibrium.

It cannot then be assumed that the gas laws apply to the partition of agglutinin between bacilli and medium, nor that the formula $\frac{C}{A} = KB^{\frac{2}{3}}$ expresses the relation, and in the application of the general formula $\frac{C}{A} = KB^n$ K and n will probably be dependent upon the conditions of the experiment, such as the manner in which serum and bacilli are brought together, the temperature, the strain of bacilli used, their age, and the constituents of the serum other than agglutinin.

Since these results agreed well with the conception of the fixation of agglutinin advanced by Bordet, I carried out a staining experiment which lends further probability to the view that the mechanisms of both have much in common. In these experiments the staining substance (fuchsin) corresponds to the agglutinin, and the bacillary substance is represented by absorbent paper.

A few drops of saturated alcoholic fuchsin were added to a litre of water. 100 c.c. of water and 100 c.c. of this solution were placed in a porcelain tray A, 100 c.c. of water and 33·3 c.c. of the solution in a tray B. A piece of thick absorbent paper of superficial area nearly equal to that of the bottom of the tray was placed in A and a similar

piece in B; the paper weighed 4·4 grammes in each case. After an interval of 24 hours a similar experiment A^1 was started, and at the same time 66·7 c.c. of fuchsin solution were added to B. The three systems were then allowed to stand a further two hours and the intensity of colour of the supernatant fluids compared. A was slightly less coloured than A^1, but B was strongly coloured. On diluting 5 c.c. of B until it had the same intensity as A^1 7·5 c.c. of water had to be added. B, therefore, contained 2·5 times the concentration of free fuchsin in A^1.

Similarly the addition of the absorbent paper in parts removed more fuchsin from the solution than in the case where the whole amount of paper was added at once.

Von Dungern (1904), and Sachs (1904), have found similar false equilibria to exist in the relations between toxin and antitoxin. Indirectly then we have further support for the view that the gas laws are not applicable to specific substances and their antibodies, and the view that we are here dealing with adsorption phenomena appears to be confirmed.

Summary.

1. The hypotheses of Pfeiffer, Emmerich and Löw, attributing agglutination to a vital paralysis due to the action of a bacteriolytic enzyme, and those of Gruber, Dineur, and Nicolle, which ascribe the action to the glutinous nature of the membranes or cilia, are insufficient to account for the observed phenomena.

2. The views of Paltauf and Duclaux that a specific precipitate is formed in the medium which mechanically carries the bacilli together are sufficient, but probably do not account fully for the agglutination of washed bacilli.

3. Arrhenius's assumption that the gas laws are applicable to the partition of agglutinin between bacilli and medium is improbable since the conditions under which these laws can be applied are absent.

4. The formula given by Arrhenius for the partition of agglutinin seems to be a special case of a general formula holding for the absorption of substances from solution by substances with highly developed surfaces, *e.g.*, the adsorption of iodine from solution by charcoal. A superficial analogy between the gas partition law and the adsorption partition law for equilibria is to be expected, likewise an equally superficial analogy between reactions in true solutions and colloidal solutions, *e.g.*, of agglutinin, for the velocity of reaction.

5. The special formula given by Arrhenius does not apply to the entire range of agglutinin solutions and the change which must be made in the constants is incompatible with the application of the gas laws, but agrees with the view that the fixation of agglutinin is due to adsorption.

6. The fixation of agglutinin from two different typhoid immune sera by living typhoid bacilli did not correspond to the partition law deduced by Arrhenius from the experiments of Eisenberg and Volk.

7. The rate of addition of bacillary suspension to agglutinating serum is a factor determining the amount of agglutinin fixed by the bacilli. By adding the suspension in parts more agglutinin is removed than in the case where the whole amount of suspension is added at once. This points to the equilibria belonging to the class met with in absorption.

8. Similar adsorption equilibria are obtained by experiments on staining.

9. The cell contents probably play an important part in agglutination as the washed membranes are but slightly agglutinated.

10. Motile cultures of *B. typhosus* grown at 37° C., and non-motile cultures grown at 42° C., agglutinated equally well, the agglutinable substance probably being unchanged.

11. The law governing the fixation in *B. typhosus*, heated to 58° C. for 30 minutes, is probably different to that holding for living cultures owing to modification of the agglutinable substance.

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THE SUCCESSFUL APPLICATION OF PREVENTIVE MEASURES AGAINST BERI-BERI.

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As the result of several years of clinical and pathological experience of beri-beri in the Malay peninsula and other parts of the Far East I have been forced to the conclusion that beri-beri is an acute infectious disease with a definite primary lesion.

The reasons which led me to this conclusion were submitted to the Government of the Federated Malay States in May, 1902, the view expressed being (1) that the disease begins after an incubation period of 10—20 days with more or less marked symptoms of gastro-duodenal irritation, often diagnosed as simple indigestion: (2) that the gastro-duodenal syndrome is accompanied or soon followed by various degrees of sensori-motor and autonomic paralysis: (3) that this syndrome lasts from 20—40 days, during which time death may ensue (acute pernicious and acute beri-beri), and that otherwise the cases either recover altogether or pass into a state of residual paralysis. I have made a large number of post-mortem examinations upon beri-beri cases, and have found that only in the acute stage of the disease autopsies showed a more or less marked gastro-duodenitis, with haemorrhagic injection of the crests of the valvulae conniventes. Microscopically a varying degree of necrosis of the mucosa was discovered, accompanied by more or less marked signs of acute inflammation and in the necrosed mucosa a special bacillus of constant morphological character was found. These changes were not observed in the residual stage of the disease; and I believe that beri-beri is thus analogous to diphtheria, in that it is an acute disease with a definite local lesion, from which a toxin is disseminated throughout the body.

Although I have not actually demonstrated that the bacillus seen in the gastric and duodenal mucous membrane escapes in the intestinal discharges, I have assumed both that this is the case and that it is by this means that the spread of beri-beri takes place.

Acting on these conclusions, I made in 1902 certain recommendations with reference to the mode of life and hygienic surroundings of the prisoners in the Kuala Lumpur gaol. More than two years have elapsed since the application of the preventive measures which I advocated was begun—time enough in which to test their efficiency. I have been unable myself to watch the effect of the new *régime,* I am therefore greatly indebted to my successor, Dr C. W. Daniels, for a report of the results, from which I have quoted freely in the following account.

The various hygienic reforms which came into force between May and September, 1902, were as follows:

(1) All prisoners were employed during the greater part of the day at extramural work. From September 23rd, 1902, they were accommodated in open-sided sheds during the day, and were thus kept out of the gaol cells from 6.15 a.m. to 4.30 p.m. The cells are thus closed for only 14 hours per diem instead of 24 hours as under the old *régime.*

(2) The whole gaol was thoroughly ventilated, all the air inlets and outlets being enlarged. The cells and corridors were in consequence not only more airy but all dampness disappeared.

(3) The gaol has been thoroughly disinfected several times. In Feb. 1902 the entire building was washed with 2 p.c. formalin. Since that time each cell in order is disinfected with 1 : 1000 perchloride solution: in this way every cell is treated once in every two or three months.

(4) Under the old *régime* the prisoners defæcated and urinated in their cells. The provision for the act was most primitive. A small box of sand was provided with a few thin sticks of wood for cleansing the anus. Observation soon showed me that the sticks were of small use and that the fingers were more often employed, and afterwards wiped on the floor or on the bedding. It appeared to me that this particular fault in the personal hygiene of the prisoners was the chief factor in the spread of the disease. In consequence on my recommendation defæcation by the prisoners in their cells has been stopped.

(5) The prisoners formerly ate their meals with their fingers, in their cells. This practice furnished a ready way in which the infecting organism might reach the intestinal tract from the dust of the cell which had been contaminated by the defective personal hygiene as

related above. This source of infection has been ended in the gaol. Since May, 1902, all meals except the evening meal at 5 p.m. are partaken of outside the cells. Since April 9th, 1904, all meals have been eaten outside of the cells.

The result of these reforms is shown in the following tables. Table I. shows the admissions for beri-beri (acute and residual) to the gaol hospital

TABLE I.

1902	Cases	Deaths	1903	Cases	Deaths	1904	Cases	Deaths
			Jan.	22	1	Jan.	—	—
			Feb.	—	—	Feb.	2	—
			March	—	—	March	8	—
			April	—	—	April*	1	—
May	90	6	May	—	—	May 1st	—	—
June	83	5	June	—	—			
July	67	7	July	—	—			
Aug.	76	2	Aug.	—	—			
Sept.	131	7	Sept.	1	—			
Oct.	99	5	Oct.	1	1			
Nov.	35	2	Nov.	5	—			
Dec.	55	3	Dec.	—	—			
Totals	636	37		29	2		11	—

* There was one doubtful case of Beriberi in April.

month by month. This table includes those prisoners who, having in previous months and years contracted acute beri-beri, still remained in a state of residual paralysis, and I do not therefore attach any great significance to these figures. That the admissions of cases in this class declined from an average of 73 per month for the period from May 1902, to Jan. 1903, to practically none (average 1·2 per month) from Feb. 1903 onwards, means that they had been cured or that they had been discharged from the gaol in due course.

TABLE II. *Early Acute Cases only.*

1902	No.	1903	No.	1904	No.
		January	6	January	0
		Feb. to Aug.	0	February	0
September	28	September	1	March	3
October	32	October	1	April*	1
November	4	November	2		
December	11	December	0		
Totals	75		10		4

* Doubtful case.

Table II. illustrates a fact of transcendent importance. It is a record of those cases of acute beri-beri which actually developed in the gaol subsequent to the initiation of the hygienic measures which I have described.

These reforms were instituted between May and September, 1902, and it is fair to assume that they became effective about September. As a result the number of acute cases fell from 28 in September, 1902, to none in February, 1903, and for seven consecutive months no fresh case occurred. Towards the end of 1903 four cases occurred; of these two had been in gaol for two months, and one for 37 days. If we admit the possibility of an incubation period of two months, it is possible that these patients contracted the disease before admission to the gaol. In the first four months of 1904 only three certain cases of acute beri-beri occurred; two of them had been in gaol for seven months and one for over a year.

It is of great importance to note that the disease disappeared from the gaol not only in the months of February to September when the dry south-west monsoon blows, but also in the months between September and March when the wet or north-east monsoon prevails. It is during the latter monsoon that beri-beri becomes almost epidemic in the public institutions and mining camps of British Malaya. It was during the latter monsoon that the disease got out of control in the Kuala Lumpur gaol before the nature of the disease was recognised and before adequate preventive measures could be devised against it. So far as this gaol is concerned therefore the preventive measures which I recommended against beri-beri were effective during that part of the year most favourable to the development of the disease.

As it might be urged that the practical abolition of beri-beri from the gaol, following the application of the hygienic measures, was but part of a general diminution of the disease throughout the Malay peninsula, Dr Daniels has obtained, through the kindness of Dr McClosky, (Table III.) a record of the admissions and deaths from beri-beri at the District Hospital of Kuala Lumpur during the period under review. The patients in this hospital are drawn from the same district as were the prisoners committed to the gaol during the same period, so that the two series of figures are fairly comparable. The table shows that, while extramural beri-beri remained epidemic, the intramural disease diminished and finally disappeared.

Table III. *Dr McClosky's Table of Admissions and Deaths at the District Hospital, Kuala Lumpur.*

1902	Admissions	Deaths	1903	Admissions	Deaths	1904	Admissions	Deaths
			Jan.	67	16	Jan.	48	17
			Feb.	61	7	Feb.	50	11
			March	61	11	March	73	10
			April	69	12	April	70	9
May	84	23	May	67	17			
June	67	16	June	80	16			
July	67	14	July	52	12			
Aug.	56	10	Aug.	57	17			
Sept.	40	5	Sept.	51	18			
Oct.	54	12	Oct.	80	12			
Nov.	55	6	Nov.	85	30			
Dec.	67	14	Dec.	66	24			
Totals	490	102		796	192		241	47

The abolition of beri-beri in this gaol has, I consider, been attained by hygienic reforms founded on the view which I have advanced that the infective agent is contained in the excreta of the patient during the acute stage of the disease and that infection results from faecal contamination. I would suggest that, as an additional preventive measure, the stools of those suffering from acute beri-beri be disinfected.

It should not be forgotten that, from May 1st, 1902, to the present time, the prisoners have been on a diet of lower grade in the nitrogen content than obtained during the previous year or so, during which beri-beri was almost epidemic. This fact should set at rest the question of diet as a factor in the causation of beri-beri.

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ON THE RELATIONSHIP OF THE PSEUDO-DIPHTHERIA TO THE DIPHTHERIA BACILLUS.

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The extreme diversity of opinion as to the identity of the Klebs-Loeffler bacillus and the bacillus of Hofmann, and the importance of the subject from the public health standpoint, make it desirable to bring forward any facts which may assist in arriving at a solution of the problem.

For some years after the two organisms were recognised their morphological and cultural characteristics served as a basis for upholding or denying any essential differences between them. In recent years, however, it has followed as a natural outcome of the study of immunity that the methods used for investigating the problems of immunity have been applied in this particular instance, since it is possible to bring about a specific reaction even in the case of closely related bodies. The more important researches carried out on these lines may be briefly mentioned.

Spronck (1896) injected large doses of pseudo-diphtheria cultures subcutaneously into guinea-pigs, and found that the local reaction produced was not influenced by the subsequent injection of diphtheria antitoxin. Glücksmann (1897) ascertained that immunisation of animals with cultures of the Hofmann bacillus did not confer any protection against diphtheria bacilli injected later. Lambotte (1902) prepared a serum which he found contained a "substance sensibilisatrice" for pseudo-diphtheria bacilli. The results of testing this serum on different strains led him to believe that these were very closely related, if not identical organisms—the specific reactions being similar in each case. He found also that there was a certain amount of fixation of this

"substance sensibilisatrice" by Klebs-Loeffler bacilli. It may be remarked that Lambotte employed the Bordet-Gengou test for the fixation of the sensitising substance.

Several observers have attempted to solve the question by means of agglutination experiments but with conflicting results. Lesieur (1901) concludes from his experiments that pseudo-bacilli do not behave otherwise than true diphtheria bacilli towards a specific serum *in vitro.* Gordon's (1903) results showed considerable variations, even in the case of different strains of diphtheria bacilli; depending on whether the bacilli used for obtaining the agglutinating serum, and those used for the tests, were recently isolated or not. Lubowski (1900) immunised a goat with an avirulent diphtheria strain. The serum agglutinated virulent diphtheria bacilli and the avirulent organism but not Hofmann's bacillus.

The experiments about to be described were performed with the purpose of determining whether substances are present in pseudo-diphtheria filtrates which, when inoculated into animals in large amounts, lead to the production of an antitoxic serum for diphtheria toxin.

The work which has been done in this direction hitherto is inconsiderable. With regard to the production of a toxin, the non-pathogenicity of Hofmann's bacillus, *i.e.* the absence of a toxin producing acute symptoms, remains in the opinion of the majority of observers its distinguishing feature. It may be noted, however, that Ruediger (1903) has described pseudo-diphtheria organisms obtained from the throats of scarlet fever patients, which proved virulent to guinea-pigs. Antidiphtheria serum did not give any protection against these bacilli. Hamilton (1904) has isolated similar organisms from the throat in various diseased conditions and on one occasion from a normal throat. On the other hand, Graham-Smith (1904) states that while investigating an outbreak of diphtheria at Cambridge he failed to meet with organisms corresponding to the type described by Ruediger. Lesieur alleges that certain strains of pseudo-diphtheria bacilli, although non-virulent for guinea-pigs in ordinary doses, can nevertheless cause fatal paralyses similar to those due to diphtheria toxin provided that large doses of recently isolated cultures be used, or smaller doses of bacilli rendered artificially active by a method devised by himself. He evidently appears to consider that the paralysing substance is a very similar body, if it is not indeed identical with the toxone in diphtheria toxin. Several years ago Salter (1899) in a communication which is

frequently quoted described some interesting experiments, apparently showing that in filtrates of the Hofmann bacillus substances exist which are identical with the toxoids present in diphtheria toxin. Cobbett (1903) states that he has repeated these experiments without being able to confirm them. Hewlett (1904) also has been unable to obtain corresponding results.

I have carried out experiments in two directions in order to test this point:—

(1) by adding varying quantities of pseudo-diphtheria filtrates to toxin-antitoxin mixtures, and

(2) by immunising horses with large quantities of the filtrates and examining the serum afterwards for antitoxin.

The cultures used comprised 11 races. No. 1 was isolated from a throat-swab which was forwarded for diagnosis. It had been sub-cultivated on serum during a long period, at least 2 years. The remaining strains were recently isolated from swabs:—

1. From nose of patient suffering from throat diphtheria.
2. Throat of same patient.
3. Diphtheritic throat.
4. Throat of a case of scarlet fever with a deposit on the tonsils diphtheritic in appearance.
5. Throat of a case of suspected diphtheria.

6.
7.
8.
9.
10. } were isolated from the throats and noses of boys from a school in which an outbreak of diphtheria occurred; the number of cases in which the Klebs-Loeffler bacilli were found was comparatively small.

All these strains had the following characters. They stained by Gram's, but not by Neisser's method. They gave an alkaline reaction to litmus when grown in glucose broth. Preparations from cultures grown for four days in alkaline broth at 36° C. when stained by Loeffler's methylene blue showed uniformly and deeply stained short rods arranged in a parallel manner. Involution forms were rarely seen even in cultures several weeks old. 5 c.c. of bouillon cultures grown for three days at 36° C., beyond slight local reactions at the site of injection, gave rise to no effects in guinea-pigs.

A large volume of culture was obtained by growing No. 1 bacillus in Erlenmeyer's flasks, each containing 200 c.c. of alkaline broth prepared in the usual manner for the production of diphtheria toxin. Another

quantity was grown in Martin's pig-stomach bouillon at 36° C. for ten days. The cultures developed a thick, opaque, slightly wrinkled membrane, of a greyish-white colour on the surface, exactly resembling that seen in diphtheria cultures; the broth was, however, somewhat turbid. The cultures were filtered through a Pasteur-Chamberland filter after growing for 10 days.

A mixed filtrate of the other 10 races was prepared by growing each separately after several sub-cultures in alkaline broth tubes. One lot was allowed to grow for nine days, the remaining flasks being filtered after 15 days' growth. Most of the cultures formed good membranes on the surface of the medium, and all had the peculiar, slightly offensive odour characteristic of diphtheria cultures.

The following Tables set forth the experiments carried out with a view to ascertaining whether toxoids were present in the filtrates. The filtrate was added first to the unit of antitoxin, the mixture being then placed in the incubator for fifteen minutes before adding the toxin. In this way any toxoid which might be present had an opportunity of combining with the antitoxin, thus preventing a corresponding amount of toxin from entering into combination.

All the mixtures were made up to the same bulk with tap-water. The toxin used was a test-toxin employed for testing antitoxic sera, the L+ dose being 0·14 and the Lo dose being 0·08 c.c.

It will be noted that in Table I the results are somewhat irregular in the case where 0·66 c.c. of toxin was given. This can doubtless be explained by the circumstance that the amount of toxin approximates so closely to the L+ dose. In the control experiment with 0·66 c.c. toxin the animal died on the 8th day, but in tests with the same amount of toxin carried out for another purpose the guinea-pig died in one case on the 4th day, and yet another on the 5th day. Differences in the resistance of different animals have also to be taken into account. With regard to the other tests in this table, although in one or two instances the animals which received the pseudo-diphtheria filtrates suffered a greater loss of weight than the controls, it is obvious that the difference comes within the limits of the errors of experiment. In Table II amounts of toxin more nearly approaching the L+ dose were selected. The results of this experiment show clearly that no evidence is forthcoming of there being any difference in the amount of free toxin present in the mixtures.

The dose of toxin given in the experiments set forth in Tables III and IV was such that toxone effects became manifest in 3 or 4 weeks.

Table I.

Testing Toxin – Antitoxin Mixtures + Pseudo-diphtheria Filtrate of No. 1 Bacillus, showing result of addition of the Filtrate to amounts of Toxin varying from a nearly L + to a nearly L° dose

Guinea-pig	Weight grams	Test	Weight of Guinea-pig on successive days after injection							
1	250	1 I. E. +0·66 c.c. T_5	255 t	240 vl	218 vl	210 vl	200 l	193 vl	195 vl	dead 22nd day
2	258	,, + ,, +1 c.c. Ps. F.	248 l	235 vl	220 vl	dead				
3	255	,, + ,, + ,,	245 m	220 vl	205 vl	185 vl	170 vl	173 vl	170 vl	dead 9th day
4	250	,, + ,, + ,,	225 l	220 vl	200 vl	dead				
5	255	,, + ,, + ,,	258 m	255 vl	253 vl	230 vl	dead			
6	255	,, + ,, +0·5 c.c. Ps. F.	230 vl	215 vl	210 vl	198 vl	205 vl	205 vl	185 vl	recovered
7	255	,, + ,, +0·25 c.c. ,,	258 m	235 vl	240 vl	225 l	215 vl	215 vl	215 vl	dead 22nd day
8	250	,, +0·64 c.c. T_5	258 s	250 vl	240 vl	230 vl	230 l	215 vl	205 vl	dead
9	255	,, + ,, +0·5 Ps. F.	255 m	260 l	250 vl	235 l	240 l	240 l	225 l	
10	250	,, + ,, +0·25 c.c. Ps. F.	238 m	230 l	228 vl	225 m	235 l	235 l	225 l	dead 22nd day
11	248	,, +0·62 c.c. T_5	265 t	242 l	230 vl	225 l	220 l	225 vl	235 vl	
12	255	,, + ,, +0·5 c.c. Ps. F.	240 l	230 vl	220 vl	220 l	215 l	215 l	205 l	
13	250	,, + ,, +0·25 c.c. ,,	240 m	235 m	238 l	245 s	240 s	230 s	220 s	
14	250	,, +0·60 c.c. T_5	240 t	240 s	225 l	215 l	220 l	215 m	215 m	
15	250	,, + ,, +0·5 c.c. Ps. F.	240 m	225 l	218 l	215 m	220 s	210 m	200 m	
16	250	,, + ,, +0·25 c.c. ,,	235 s	225 m	235 l	235 m	235 m	230 s	220 s	
17	255	,, +0·56 c.c. T_5	245 n	240 s	225 l	220 l	220 m	220 t	215 m	
18	250	,, + ,, +1 c.c. Ps. F.	215 m	200 l	195 vl	195 l	200 m	200 m	195 s	
19	245	,, + ,, +0·5 c.c. ,,	230 s	220 l	220 m	225 s	225 s	220 t	220 s	
20	245	,, +0·52 c.c. T_5	245 n	230 t	228 s	215 m	215 m	215 t	215 t	dead 11th day
21	250	,, + ,, +1 c.c. Ps. F.	245 s	228 m	235 s	235 s	225 t	220 t	223 n	
22	250	,, + ,, +0·5 c.c. ,,	240 t	230 s	225 t	228 t	225 t	220 t	228 t	
23	260	,, +0·50 c.c. T_5	258 n	255 t	265 t	245 t	260 t	255 t	255 n	
24	248	,, + ,, +1 c.c. Ps. F.	240 m	230 m	225 t	235 t	230 t	220 t	215 t	
25	255	,, + ,, +0·5 c.c. ,,	275 t	250 n	235 t	235 t	240 n	240 n	255 n	

Note:—1 I. E. = 1 unit of Antitoxin (Ehrlich). T. = Toxin. Ps. F. = Pseudodiphtheria Filtrate.

The Toxin was diluted 1 : 5 with water for convenience of testing; the amount of Toxin represents amounts of this dilution in this and the following Tables.

The letters "n," "t," "s," "m," "l," and "vl," refer to the size of the local reaction, and indicate respectively that there was no local reaction; a trace of reaction; and a small; medium; large; and very large reaction.

TABLE II.

Testing Toxin – Antitoxin Mixtures + Pseudo-diphtheria Filtrate (10 races grown separately and Filtrates mixed), showing result of addition of the latter to amounts of Diphtheria Toxin approaching the L + dose.

Guinea-pig	Weight grams	Test	Weight of Guinea-pig on successive days after injection							
1	275	1 I. E. + 0·69 c.c. T_5	260 l	250 vl	230 vl	230 vl	—	dead	—	—
2	260	,, + ,,	260 m	255 l	230 l	225 vl	dead	—	—	—
3	255	,, + 0·67 c.c. T_5	260 m	250 l	230 l	210 l	dead	—	—	—
4	250	,, + ,,	240 m	235 vl	210 vl	215 vl	—	180 vl	dead	—
5	265	,, + 0·69 c.c. T_5 + 2·5 c.c. Ps. F. 9 days	255 l	255 vl	225 vl	225 vl	—	dead	—	—
6	260	,, + ,, + ,, ,, 15 days	265 m	270 vl	250 vl	250 vl	—	215 vl	dead	—
7	255	,, + 0·67 c.c. T_5 + 2·5 c.c. Ps. F. 9 days	250 m	250 vl	255 vl	250 vl	—	250 vl	250 vl	265 vl
			Paralysis pronounced on 28th day; dying on 33rd day; dead next day.							
8	250	,, + ,, + ,, ,, 15 days	230 m	215 vl	190 vl	180 vl	—	dead	—	—

Table III.

Testing Toxin – Antitoxin Mixtures + Pseudo-diphtheria Filtrate (mixed Filtrates of 10 races), showing effect of addition of latter to amounts of Toxin representing paralysing dose.

Guinea-pig	Weight grams	Test	Result
1	275	1 I. E. + 0·65 c.c. T_5	Died with paralytic symptoms on the 28th day.
2	265	,, + ,, ,,	Marked paralysis on 30th day, but gradually recovered thereafter.
3	270	1 I. E. + 0·65 c.c. T_5 + 2·5 c.c. Ps. F.	Marked paralysis on 23rd day; died next day.
4	260	,, + ,, + ,, ,,	Paralysis on the 23rd day; died on 28th day.
5	250	,, + ,, + ,, ,,	Died on 23rd day.

Table IV.

Repetition of Experiment given in Table III.

Guinea-pig	Weight grams	Test	Result
1	260	1 I. E. + 0·65 c.c. T_5	Died on 8th day. P.M. Toxaemia.
2	260	,, + ,,	Showed commencing paralysis on 16th day, which became very pronounced on 21st day; died on 24th day.
3	260	,, + ,,	Showed marked paralysis on 20th day; died on 21st day.
4	250	,, + ,, + 2·5 c.c. Ps. F.	,, ,, ,, ,, 24th day; ,, 28th day.
5	250	,, + ,, + ,, ,,	,, ,, ,, ,, 22nd day; ,, 24th day.
6	250	,, + ,, + ,, ,,	Died on the 11th day.

The results are not conclusive of any marked difference in the paralytic effects produced.

Taken as a whole these experiments may be interpreted as indicating that no toxoids were present in the filtrates of the pseudo-diphtheria bacilli examined.

Immunisation with Filtrates of the Pseudo-Diphtheria Bacilli.

Another method of testing the point was tried. If toxoids be really present as a product of metabolism of the Hofmann bacillus it might be expected that immunisation of a suitable animal with their products ought to lead to the production of an antitoxin in the animal's serum. The validity of this contention will be discussed later.

The process of immunisation was carried out in the following way:

A horse "A" which had not been previously immunised, received doses of pseudo-diphtheria filtrate of No. 1 bacillus, the procedure being exactly the same as in a diphtheria immunisation. Beginning with 3 c.c. it received as a final dose 5 weeks later 1 litre; 3,200 c.c. being given altogether. Before the injections were begun the serum of the horse was tested for normal antitoxin. It was found that it did not contain 1/3 unit per c.c. Ten days after the final injection it was again tested and again was found not to contain 1/3 unit.

A month or two later this horse received 1/10 c.c. of a diphtheria toxin, the M.L.D. of which for guinea-pigs of 250 grammes was 1/100 c.c. No further injections were given until 10 days later. A sample was taken on this day and the serum was again tested. It was found to contain .1 unit per c.c. At the end of the ordinary diphtheria immunisation the antitoxic value of the serum was low (below 100 units per c.c.) and in subsequent immunisations never rose above 400 units per c.c. It may be noted that the morning after a dose of 450 c.c. of the filtrate had been given the horse showed a considerable local reaction.

A second horse "B" was treated in a similar manner. He received 2150 c.c. of the mixed filtrates (10 races grown for 15 days). Two days after the last injection of 1 litre, a litre of the 9 day filtrate was given. The period of immunisation extended over 20 days. Ten days later a sample was taken. Before the injections were commenced the serum was tested and was found to contain no normal antitoxin, *i.e.* not 1/4 unit per c.c. The serum of the sample taken after the immunisation also did not contain 1/4 unit per c.c. The horse was then

given 1/100 c.c. of a diphtheria toxin whose M.L.D. for guinea-pigs was 1/400 c.c. After an interval of 10 days, blood was withdrawn, and the serum again found to have less than a 1/4 unit per c.c. Four days later 1/20 c.c. was given intramuscularly. It was intended to obtain blood for the purposes of a test at the end of 10 days, but unfortunately the horse died suddenly; the cause of death apparently having no connection with the previous treatment.

On the evening after 150 c.c. of the filtrate was administered the horse had a temperature of 104·4° Fahr. and a small swelling the size of the fist over the site of injection. After 300 c.c. he had a local reaction with an oedematous swelling in the brisket and stiffness in walking. The dose of 1 litre of the 15 days' filtrate caused a moderate local swelling accompanied by oedema in the brisket. The symptoms, however, were not nearly so severe as those usually observed during a diphtheria immunisation.

A third horse "C" was inoculated with 650 c.c. of the 15 days' filtrate given by rapid stages, and as a final dose, 1,100 c.c. of the nine days' filtrate. The period of immunisation lasted only nine days. The serum before beginning the treatment did not contain 1/4 unit, and ten days after the last dose was given, again did not have an antitoxic value of 1/4 unit per c.c. The horse was then injected on successive days with 1/100, 1/50, 1/10, 1/2, 1·25 and 3 c.c. of a toxin whose M.L.D. for guinea-pigs was 1/300 c.c. The blood was sampled after an interval of ten days, and the serum was found to contain 1 unit per c.c., but not five units. In the evening after 150 c.c. of the pseudo-diphtheria filtrate had been given the horse had a moderate local reaction. He felt the last dose (1,100 c.c.) considerably, fed badly for a few days, and had a large local reaction.

It is evident from these experiments that the filtrates employed were not capable of producing an antitoxin to diphtheria toxin. Before the conclusion is drawn that no toxoids were present in the filtrates the question must be faced as to whether toxoids alone can give rise to an antitoxin. At the Thirteenth International Congress of Medicine at Paris in 1900 Ehrlich made several observations bearing upon this point which leave no doubt that he believed that they are able to do so. While referring to his own views on the constitution of diphtheria toxin he stated that it was possible to provoke a production of an antibody not only by utilising toxins but also toxoids. As a result of later investigations in conjunction with Morgenroth on the production of isolysins Ehrlich (1900) found that before such an antibody could be

obtained very large quantities of blood must be injected; the reason being that in the blood of the same species elements corresponding to toxophore groups are absent. He considers that what he calls an "ictus immunisatorius" is essential. Wassermann at the International Congress of Hygiene at Brussels in 1903 discussed the matter at some length. His conception of the mechanism of antibody-production may be briefly stated thus:—He asserts that he has never succeeded with quite non-toxic "toxins," *i.e.*, toxins with haptophore groups only, in obtaining a really high antitoxic serum. In order to bring about an "Abstossung" of the receptors a second factor besides the combination of the haptophore groups to the cells is necessary, viz., the stimulus (Reiz) to the cell which is the function of the toxophore groups. He points out, however, that the possibility of immunising with toxoids is beyond question, and gives as an example the basal immunity conferred on guinea-pigs and mice against tetanus by using non-toxic modifications: the only possible method indeed of producing an immunity in these animals against diphtheria and tetanus toxins. Bruck (1904) in a recent paper describes experiments which he thinks strengthens the position taken up by Wassermann. He obtained two tetanus toxins, one practically non-toxic for mice, and the other feebly toxic. He proved the presence of toxoids in these toxins by finding that they were still able to neutralise antitoxin. He then proceeded to immunise rabbits, giving doses up to 1 c.c. of each. In the case where the non-toxic substance was used no antitoxin was produced, while in the other case, a small amount of antitoxin was found in the serum of the rabbit. The objections can, I think, be reasonably adduced that the total quantities of the toxins given were very small,—Ehrlich's isolysin experiments may be recalled in this connection,—and that the rabbit is not perhaps a very suitable animal for the production of an antitoxin. Bruck believes that a slight stimulus is necessary in addition to the action of the haptophore groups for the production of an antibody.

Von Behring's (1904) views on this subject are founded on a very large practical experience. In immunising horses in order to obtain tetanus antitoxin he now uses toxins whose "direct toxic value" has almost, if not completely disappeared, but which have retained their "indirect toxic value," *i.e.*, their power of neutralising antitoxin. He refers to a tetanus toxin which, although quite non-toxic for mice, can be used for immunising horses without the slightest risk, and which in a short time confers upon them a high degree of immunity with a considerable production of antitoxin. Before he recognised this fact he had

empirically adopted the method of adding trichloride of iodine to his toxins in order to bring about a similar result.

The weight of von Behring's authority on this point, together with the fact that Wassermann does not deny that a small amount of antitoxin can be produced by toxoids alone, is confirmatory of the belief that no toxoids were present in the pseudo-diphtheria filtrates used in the immunisation experiments described above. This view gains support from the circumstance that after a small amount of diphtheria toxin was given the antitoxic values of the serum of the horses "A" and "C" were not above the average. It might have been supposed, otherwise, that the receptors produced in abundance by toxoids but remaining attached to the cells would have been set free in the serum when a stimulus provided by even a small dose of toxin was supplied.

In conclusion it may be stated that the two sets of experiments carried out combine to justify the opinion:

(1) that no substances capable of neutralising diphtheria antitoxin are present in filtrates of pseudo-diphtheria bacilli;

(2) that the results of the immunisation of horses with large quantities of the filtrates make it apparent that they do not contain substances capable of stimulating the production of an antitoxin to diphtheria toxin.

It is scarcely necessary to add that if this be the case the differences between the two organisms are accentuated, thereby diminishing the probability that they stand in a close relation to each other. The whole question has a certain interest clinically, since it shows that in cases where Hofmann's bacillus is associated with the Klebs-Loeffler bacillus no toxoids are elaborated by the former which might do harm by combining with antitoxin administered therapeutically.

My thanks are due to Dr Fletcher, Ham Green Hospital, Bristol, and to Dr A. T. MacConkey, for supplying me with the material from which the cultures were isolated. I am also indebted to Dr George Dean for practical suggestions which have been of value in carrying out the experiments.

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BACTERIOLOGICAL EXAMINATION OF TIDAL MUD AS AN INDEX OF POLLUTION OF THE RIVER.

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INSPECTION by a sanitary expert of all tidal rivers where oysters or other shell-fish are laid, or found, is of the highest importance, but it may frequently, with advantage, be supplemented by a bacteriological investigation.

Early in 1903, when my attention was drawn to the subject, I recognised that the bacteriological examination of the shell-fish alone might readily lead to misleading results owing to the probable want of uniformity of the pollution, if such had taken place, and so unequal distribution of the bacteria which are taken as the index of such pollution. At first I hoped that sampling the sea or tidal water over the beds would give more uniform results, but increased experience showed me that such samples were also liable to error and irregularity of results.

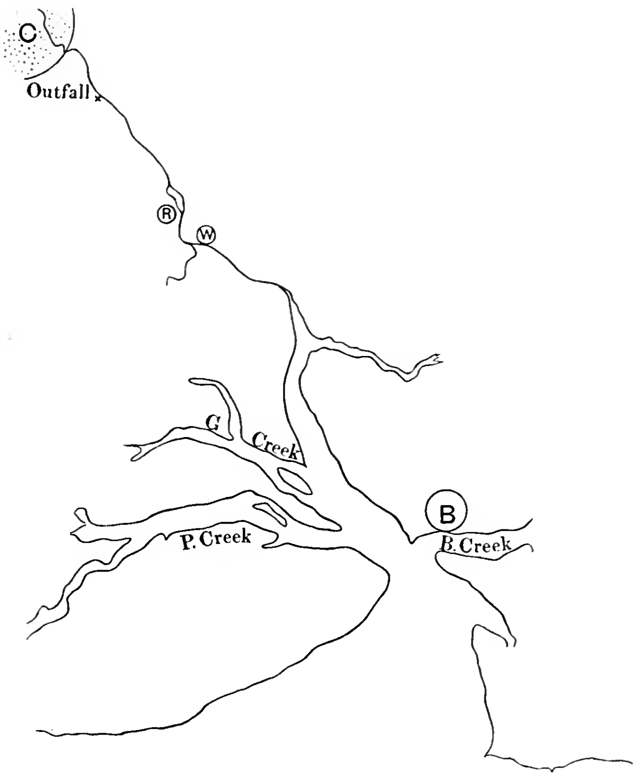
The Royal Commission on the treatment of disposal of sewage in their 4th Report drew attention to this matter. They state (p. 33) "it is clear that a single examination of chance samples of oysters might be most misleading as to the bacterial flora of the whole oyster-contents of a laying or pond," and remark that a similar caution is necessary in the case of waters. The bacterial content of the waters must obviously differ considerably according to the tide and especially whether ebb or flood water.

On theoretical grounds it seemed to me probable that the examination of samples of the river mud would yield results more uniform and more reliable; and it might well be possible from a bacteriological examination of a tidal mud to indicate how far such a position was suitable or unsuitable for shell-fish layings.

The following investigation deals with the results of a series of examinations of mud from a tidal river which has extensive oyster beds in creeks opening near the mouth of the river and beds also in the mouth of the river. About 8 miles from the mouth is a town of 40,000 inhabitants, which discharges its sewage into the river about a mile lower down. The sewer outfall is rather over 7 miles from the mouth of the river (see Map).

The only sources of pollution of this stretch of tidal river are as follows:

(1) The sewage of the town C. This is discharged into the river as above stated, but after treatment, while the plant is sufficiently large to enable the effluent to be discharged only on an ebb tide. The method of treatment consists of sedimentation in six large settling tanks followed by bacterial treatment in coke breeze and clinker beds (contact beds). The average amount of sewage dealt with is about 1,000,000



gallons per day. The sludge from the sedimentation tanks does not run into the river, but is pressed into cakes and deposited on the land.

The effluent has always been clear when I have examined it and will keep indefinitely without putrefactive odours developing.

(2) From a village (R on map) about $1\frac{1}{2}$ miles below the sewer outfall of the town C small drains discharge into the river. Also $\frac{1}{4}$ mile below R but on the opposite side of the river, the drainage of another small place (W on map) with a population of 2,600, passes into the river.

Both W and R, however, have very few water-closets, and most of the privy contents finds its way directly on to land far away from the river.

(3) The drainage of the small town B (population about 4,600) is discharged into the mouth of the river by means of a single outfall, on a point 110 feet from the shore but above the low-water mark. The position was selected for the outfall after float experiments, which showed that the sewage effluent was conveyed out to sea by the currents and tides.

The sewage flows by gravitation into a precipitation tank, capacity 216,000 gallons, where it is treated with alumino-ferric. The sludge is disposed of on land, while the effluent is discharged into the sea during ebb-tide only, the valve being opened one hour after high water and closed one hour before low water. There is a storage tank capable of holding 100,000 gallons. This outfall is separated by the whole width of the estuary of the river from the creek P.

These are the only sources of sewage pollution. The oysters in creek P have been stated by Dr Bulstrode to be free from risk of pollution. Into creek P no drains discharge.

It must be clearly understood that the investigation here recorded was not undertaken to see if this particular river showed evidence of pollution or not.

The river is one the topographical characters of which have been repeatedly investigated by competent authorities and may be considered well known.

The main object was to see if the bacteriological examination of tidal mud from different parts would bear out the results obtained by careful topographical investigation, and how far such bacteriological mud examination may be taken as a reliable indication of the degree of pollution.

On topographical grounds the river may be divided into four parts.

I. *Obviously and markedly contaminated.* From the town of C to say a mile below the sewer outfall of this town.

II. *Considerable but less marked contamination.* From where I leaves off to, say, $1\frac{1}{2}$ miles below township W.

III. *Slight or at least very diluted contamination.* The rest of the main channel of the river down to the mouth.

IV. *Free from any but extremely diluted sewage contamination.* The creeks P and G.

Here the water passing down the river does not at any time enter these channels for they are at the same time discharging their contents.

No drains run into them and the only sewage which can enter must be that which has passed out to sea, mixed with the many million gallons of pure sea water and then, if any, returned with the incoming tide.

Do the bacteriological results confirm these topographical considerations?

A number of samples were taken at different times, the results of which are set out in Tables I to IV.

The tables correspond to the topographical groups set out above.

Bacteriological methods of investigation.

In order to measure bacteriologically the degree of contamination it is necessary to have some reliable indication of contamination. The total number of bacteria present per gramme would probably be of but little service. A considerable experience with drinking-water, soils, etc. has convinced me of the especial value of *Bacillus coli* as such an indicator.

It is found in sewage and animal excretion in very large numbers, while there is no reliable evidence to show that it is found, or found in any but very small numbers, in soil, mud, or water which has not been contaminated by sewage or animal excretion.

The value of streptococci (as a class) has been less certainly and conclusively determined, but many workers (notably Houston) urge their great importance as a means of estimating excretal pollution.

The number of *B. coli* and of streptococci per gramme were taken therefore as the gauge of the amount of excretal contamination, the greater stress being laid upon the number of *Bacillus coli.* The number per gramme were determined in every case.

The samples were collected in sterile glass-stoppered bottles, and were in most cases examined within a few hours of collection; for a few the examination was made early next day. The state of the tide was recorded in every case, but it apparently made no difference to the bacterial content of the samples.

The same method of sampling and examination was used throughout so that the results should be strictly comparable.

1 grm. of the mud was carefully added by means of a sterile spatula to 9 c.c. of water in a 2 oz. wide mouth bottle (Kali pattern), fitted with a solid indiarubber cork, the whole bottle and contents being of course sterile. The indiarubber cork enabled the sample to be very thoroughly mixed. Then 1 c.c. of the diluted mud (labelled Dilution *A*), again well mixed *immediately* before the 1 c.c. was abstracted, was added to 99 c.c. of sterile water in a flask and labelled Dilution *B*. From *B* in the same way 1 c.c. was added, after intimate mixing to 99 c.c. of sterile water in a fresh flask (Dilution *C*). 0·1 and 1·0 c.c. of each of the dilutions were added to tubes of MacConkey's taurocholate medium and to neutral-red broth respectively. With muds where previous experience had shown the relative absence of *B. coli* and streptococci it was not necessary to also inoculate tubes from dilution *C* in every case. For *B. coli* MacConkey's medium was used. Neutral-red broth, though very valuable for *B. coli* detection in drinking-water, is in my experience much less suitable for sea waters and muds. The characteristic reaction is not always obtained, while other reducing organisms are sometimes present. The tube showing a positive result (gas and acid) with the greatest dilution was examined for *B. coli*, the organism being *in every case* isolated and its characters worked out. For the preliminary plating to isolate, Drigalski and Conradi's medium was used in the majority of cases. A positive result was not recorded unless a typical *B. coli* was isolated.

For streptococci the dilutions (0·1 and 1·0 c.c. of *A* and *B* and *C*) were added to tubes of neutral-red broth. These were incubated at 37° C. for 40–48 hrs. and then examined in hanging drops for streptococci. Frequently examinations after 24 hours' incubation were made in addition, but in every case hanging drop preparations were made after 40–48 hrs. I found the 40–48 hours' examination the best, as in no case did I find streptococci in 24 hrs. or 3 days in dilutions which did not show streptococci after 40–48 hrs., while the converse was not always the case. Several hanging drop preparations were always made from the dilution below the one giving positive results. No attempt was

made to isolate the streptococci, although I satisfied myself in every positive case that streptococci were present. These results must therefore be taken as indicating streptococci as a class, and do not deal with the different varieties present.

If *B. coli* or streptococci were found in 1 c.c. of Dilution *A* it was assumed that at least 10 per grm. of the mud were present and so for the other dilutions.

1 c.c. $A = 0{\cdot}1$ grm. mud; 0·1 c.c. dilution $A = 0{\cdot}01$ grm.;
1 c.c. dilution $B = 0{\cdot}001$ grm.; 0·1 c.c. dilution $B = 0{\cdot}0001$ grm.;
1 c.c. dilution $C = 0{\cdot}00001$ grm.; 0·1 c.c. dilution $C = 0{\cdot}000001$ grm.

Consideration of Tables I to IV.

Table I gives the results of samples taken from mud which on topographical grounds should be markedly polluted. Excluding No. 10 taken above the town C all the 10 samples show *B. coli* present in the proportion of at least 10,000 per gramme, while the number of streptococci present was never less than 100 per gramme.

In Table IV on the other hand the samples are from sources not subject to any but remote contamination. Here out of 8 samples *B. coli* numbered in one instance over 100 per gramme, but in the other samples 10 or less than 10 per gramme; while for streptococci the results showed 10 or less than 10 per gramme.

The contrast between these two sets of results is sharp and uniformly present.

The contamination of the samples in Table II would be only slightly less than in Table I, since the diminished contamination from the sewage of C owing to increased distance from the point of fouling would be partly compensated by the added pollution from the drainage of R and W. The bacteriological results of Table II bear this out, since they are but slightly better than those of Table I.

In the third group the self-purification factors and the much greater dilution of the sewage would cause the liability and possibilities of pollution to be vastly diminished; and the results of Table III show a marked improvement, *B. coli* being 100 per gramme (*i.e.* strictly speaking over 100 but less than 1000), and streptococci 10 per gramme.

A few samples were also taken from Creek B. These have a special interest of their own apart from the general interest of the inquiry.

The untreated sewage of the town B used to be discharged by three separate outlets on to the foreshore of the creek. Since 1899 the

sewage has been chemically treated and discharged at a single outfall at the junction of creek and river. It is discharged only at ebb tide and when it can be carried directly out to sea. In the creek there is also a considerable amount of traffic, boats being laid up in it during the winter.

The results of the five examinations (see Table V) are very unequal, as might be anticipated. Only near the ferry is there marked evidence of pollution. At the other parts of the creek a very considerable state of purity is evinced. Unfortunately no results are available for comparison with samples of mud taken before the sewage was diverted and treated.

The figures show that the purification effected has been most considerable, and, now that sewage no longer gains access to the creek, the mud compares very favourably with that from the main river.

These results I think clearly indicate that the examination of tidal mud gives valuable results, which closely accord with topographical data.

Before the full value of the bacteriological examination of tidal mud as a reliable measure of the actual amount of sewage or animal contamination can be gauged there are several obvious possibilities which must be considered.

In the first place do such mud examinations yield *uniform* results? The two factors which might be considered as possibly causing widely varying results are, in the first place, the influence of temperature or season, and secondly the irregularity caused by the contaminating material not being uniformly distributed but deposited locally and irregularly.

With regard to the first—the influence of temperature or season—samples were taken on purpose at very different times of the year. In the tables the date of collection is given in every case. A consideration of the tables does not show any marked variation due to season. Thus, for example, in Table II, samples 7 and 21 were taken from the same place as far as possible, one sample in February and one in August. Identical results were obtained both for *B. coli* and for streptococci.

With regard to irregularities of deposition of sewage-contaminated matters, on theoretical grounds this might be considered likely to occur, but in view of the constant agitation of the mud such irregularities of distribution would probably be quickly remedied. This point was however experimentally considered. Thus the three samples 23, 24, 25, were purposely taken as follows: No. 23 was collected about $\frac{3}{4}$ mile

below the sewage outfall of C from the left bank, tide coming in and about $2\frac{1}{2}$ hours after low water; No. 24 was collected on the same side but 100 yards above No. 23; and No. 25, about 100 yards above No. 24. All three samples were collected at the level of the water and immediately after one another. Identical results were obtained for both *B. coli* and streptococci, showing a remarkable uniformity of contamination so near the sewer outfall and over an area of about 200 yards.

Six other samples were specially collected from the creek P with this question in view. The results are shown in Table VI, but not in Tables I to IV. Here samples *a* and *d* were from the same place as regards distance from the mouth of the creek, but sample *a* almost at low water, and *d* at half-flood tide, in both cases at the level of the water. In the same way for samples *b* and *e*, and samples *c* and *f*, except that *b* and *e* were taken 100 yards further up the creek than *a* and *d*, and *c* and *f* 100 yards higher up still than *b* and *e*. It will be seen that almost identical results were obtained, five being identical and the sixth only differing in that *B. coli* were present in the proportion of 10 (less than 100) per gramme, while this organism was not found in $\frac{1}{10}$ gramme in the other samples.

I do not wish—and with so few samples it would not be justifiable to do so—to attach too much value to these figures, but as far as they go they certainly show marked uniformity, in striking contradistinction to results not infrequently obtained with the tidal sea water. Unreliability from local irregular contaminations seem to be, in the main, eliminated in mud samples. The constant stirring up of the mud would make for uniformity.

Another question which must be faced deals with the question of the time and age of the pollution. In other words, will tidal mud be a good index of present and recent contamination, or do the results merely show that contamination has taken place at some antecedent —perhaps long antecedent—period?

This consideration is obviously an important one, since if the detection of large numbers of *B. coli* and streptococci in a mud sample merely means that it has been polluted possibly at a very remote period, much of the significance of their detection is lost. For instance, if a mud is washed with a sea water comparatively pure, and containing let us suppose only *B. coli* and streptococci in 10 and 100 c.c. respectively, then these organisms will sink and contaminate the mud, and if they do not die out even a pure mud washed by a comparatively pure sea water, would in time yield high figures as regards *B. coli* and streptococci.

The results of Tables I to IV would show that this can scarcely be the case, while experiments of other workers have not demonstrated any multiplication of *B. coli* in mud. I am not acquainted with any direct experiments in this connection with regard to streptococci in tidal mud. I have, however, made a number of direct experiments, since on other grounds as well—notably the question of the sensitiveness of mud examinations as indicators of degree of contamination—the matter merits further consideration.

My results may be recorded under two divisions:

A. Experiments with moist muds kept in stoppered bottles.

B. Experiments with muds kept as nearly as possible under natural conditions.

Series A. Four muds were in this way kept in stoppered bottles and reexamined after an interval of a week or more.

The results are shown in Table VII.

This table shows that with all four muds the number of streptococci per gramme rapidly diminished, and that the number of *B. coli* also decreased.

In no case was there any observable increase on keeping, of either *B. coli* or streptococci.

These muds however received no sea water and could not be considered as kept under natural conditions.

Series B. In this series several samples of mud were kept in open tanks in a shed. Several pounds of mud were used for each experiment, and each mud sample received twice a week $1\frac{1}{2}$ gallons or more of fresh sea water, the mud and water being thoroughly mixed after each addition. The mixture was then allowed to stand until the next sample of sea water was received. The supernatant sea water was then carefully siphoned off, and the fresh sea water added and thoroughly mixed. The tanks were kept in a shed, the temperature of the water and of the air being carefully recorded each morning.

Here the conditions approximated to a certain extent to those met with in nature.

In order to obviate the risk of added *B. coli* and streptococci in the sea water, for most of the experiments of this series, the sea water was partially sterilized in the steam sterilizer and heated up until steam was evolved. The water was then removed and rapidly cooled by standing the vessels in cold water.

Tank I. Mud collected Aug. 30, 1904, from the river about $\frac{1}{4}$ mile below the sewage outfall pipe. Put into the tank, and sea water added

Aug. 31st. The sea water was added twice a week and varied from $1\frac{1}{2}$ to $2\frac{1}{2}$ gallons at a time. In every case it was partially sterilized as above. The examinations were made in the same way as for samples collected under natural conditions. The results of the examinations are given in Table IX.

Tank II. Mud collected from the river about 80 yards below the sewage outfall. Collected Sept. 12th, 1904, and put into the tank and started within a few hours of collection. Experiment exactly as for Tank I, except that at first the sea water was added unheated or untreated in any way. On and from Oct. 25th the sea water was partially sterilized exactly as for Tank I. The results of the examinations are given in Table X.

Tank III. Mud collected from the river Oct. 20th about $\frac{1}{2}$ mile above the sewage works. Put into the tank and sea water added next day. Partially sterilized sea water added biweekly exactly as for Tank I. The results of the examinations are given in Table XI.

Temperature during the tank and other experiments. The temperature of the air of the shed and of the water was recorded every morning at 9.0 a.m. throughout the experiments, *i.e.* from Aug. 31st to Dec. 14th, 1904. I do not think it necessary to burden the paper with these temperatures in detail. The following data will give a general idea of the temperature variations. Air temperatures from Aug. 31st to Sept. 9th, 13°–15° C., Sept. 10th to 12th 11°–12° C., Sept. 13th to 19th 13°–15° C., Sept. 19th to Oct. 6th 11°–13° C., Oct. 7th to 16th 5°–12° C., Oct. 19th to 24th 12°–15° C., Oct. 25th to Nov. 11th 9°–11° C., Nov. 12th to 17th 5°–8° C., Nov. 18th to 21st 0°–4° C., Nov. 22nd to 29th 7°–9° C., Nov. 30th to Dec. 6th 3°–6° C., Dec. 7th to 13th 0°–4° C.

These are air temperatures, the temperature of the tank water was usually 1–2 degrees lower.

As, for part of the experiments, fresh unheated sea water was added to the tanks it is of interest to have some idea of the bacterial content of the sea water, which was obtained by rail from the G. E. Railway. Six bacteriological examinations were made. The following is a summary of the results as regards the numerical presence of *B. coli* and streptococci.

Examined Sept. 13th. *B. coli* present in 10 c.c. not in $\frac{1}{10}$, $\frac{1}{2}$, 2 c.c.; streptococci not in 12 c.c.; larger amounts not examined, *B. coli* isolated quite typical.

Examined Sept. 27th. *B. coli* absent in $\frac{1}{10}$, $\frac{1}{2}$, 2, 10 c.c.; no streptococci in 50 c.c.

Examined Oct. 4th. *B. coli* in 2, 10, 40 c.c., smaller amounts not examined. *B. coli* isolated, typical, except that no milk coagulation (3 weeks' incubation: repeated same result). Streptococci in 10 and 40 c.c. smaller amounts not examined.

Examined Oct. 14th. *B. coli* absent in $\frac{1}{10}$, $\frac{1}{2}$, 2, 10 c.c. streptococci present in 10 c.c., smaller amounts not examined.

Examined Oct. 21st. *B. coli* present in $\frac{1}{2}$, 2, 10 c.c. Quite typical characters. Streptococci in 10 c.c., less not examined.

Examined Dec. 7th. *B. coli* and also streptococci absent in 2 and 10 c.c., larger amounts not examined.

It will be seen that the bacterial content, as regards *B. coli* and streptococci, of the sea water supplied, varied very considerably.

Examinations of Tables IX, X, and XI, show on the whole very similar results for all three tank experiments.

All three gave identical results when started, *i.e.* *B. coli* greater than 100,000 but less than 1,000,000 per gramme, and streptococci greater than 1,000 but less than 10,000 per gramme of mud.

A gradual, but not perfectly regular, decline takes place week by week in the number of both *B. coli* and streptococci.

The decline for Tank II which up to Oct. 25th received unheated sea water, containing living *B. coli* and streptococci often, is naturally slower than for Tank I.

The results obtained show that streptococci usually diminish and die out much more rapidly than *B. coli* and are thus indicators of more recent contamination of tidal mud. Tank III results indicate however that some forms of streptococci are apparently very resistant. From my whole series of experiments I should however conclude that these are comparatively rare in tidal mud.

B. coli diminish in numbers, at first fairly rapidly, but when the number is reduced to 100–1000 per gramme, the decline is comparatively slow. Further, although with time, markedly greater diminution does take place, yet even in muds kept under these, as far as possible, natural conditions the *B. coli* do not quite disappear, but are found in the proportion of at least 1 per gramme, and this after the lapse of three months from the initial pollution.

These experiments emphasise the great importance of the *enumeration* of *B. coli* in estimating pollution. The mere detection of *B. coli* is valueless. Here are muds in which after the lapse of three months (during which two at least (Tanks I and III) have received no fresh

B. coli), this organism can still be found, if one gramme of the mud be examined.

On the other hand when the *quantitative* aspect is considered, it is seen that there is a vast difference between the freshly contaminated mud with more than 100,000 *B. coli* per gramme, and the mud not polluted for three months, with but 1–10 *B. coli* per gramme.

The tank experiments, in my opinion, confirm the view that tidal mud is a good index of present and recent pollution of the river when the results are properly interpreted.

They indicate that the examination of tidal mud will not only show that pollution has taken place, but will give some indication as to the time and amount of such pollution.

They further show that the presence of *B. coli* in 0·1 gramme of mud cannot be considered as indicating recent contamination, unless by material itself very slightly polluted.

For Tank I, 6 weeks after the experiment was started, which may be considered as 6 weeks after the pollution of the mud with sewage, *B. coli* were still found in 0·1 grammes: for Tank II this organism was present in the same amount after 9 weeks: while for Tank III 5½ weeks after the pollution *B. coli* were still present in 0·01 gramme.

No doubt however under quite natural conditions, and with a washing of the mud at each tide with a pure sea water, a more uniform and rapid decline in the number of both *B. coli* and streptococci would have taken place.

It is of interest to notice that these laboratory results are quite in accord with the samples taken from the river, as given in Table IV. The results given in Table IV show that these muds which on topographical grounds are certainly free from pollution, except of remote and extremely diluted kind, almost constantly contained *B. coli* to the number of at least 10 per gramme, but only in one case to the number of at least 100 per gramme.

The question of specific pollution with the typhoid bacillus of tidal water or tidal mud must also be directly considered.

Are we in a position to say from the results of the examination of tidal mud, *e.g.* from an enumeration of *B. coli* and streptococci, that the typhoid bacillus is certainly absent from such tidal mud?

In the first place it will I think be conceded that if typhoid bacilli do gain access to a tidal river they will almost certainly be mixed with a preponderating number of *B. coli* and probably also with streptococci more numerous than themselves. If the pollution is by *urine*, infected

with the typhoid bacillus, this may not be the case, but such an infection must be a very exceptional one, and in general, specific pollution with typhoid bacilli means also a concurrent pollution with vast numbers of *B. coli* and streptococci. In other words, the muds of Tanks of I, II, and III might readily be muds also specifically polluted with the typhoid bacillus.

How long is this latter organism likely to survive in such muds, or put in another way, what numbers of *B. coli* and streptococci are sufficient to enable us to say with certainty, or at least practical certainty, that such a mud, and so presumably any shell-fish in it, is free from typhoid bacilli?

It is usually freely assumed that *B. typhosus* will die out more rapidly than *B. coli*, and probably this is true, but no exact experiments have been made, to my knowledge, with tidal mud.

I have therefore made these questions the subject of a number of direct experiments.

Group I. B. typhosus in sterile moist tidal mud.

If the typhoid bacillus died out rapidly in sterile mud the question would be readily answered whether results with regard to *B. coli* and streptococci are sufficient to indicate absence of the typhoid bacillus.

Two experiments were undertaken to estimate the vitality of *B. typhosus* in sterile mud. In most of the experiments published in regard to the vitality of this organism in sterile fluids, only the presence or absence of the organism has been usually determined. Here quantitative estimations were made so that the rate of increase or decrease could also be studied.

Exp. 1. Mud collected from the river and from a source similar to No. 22, Table II: Mixed with a little sea-water and sterilized in a flask with cotton-wool stopper for 45 minutes at 115° C. in the autoclave. The mud was of such consistency that after mixing well, it could be drawn up into an ordinary sterile graduated 1 c.c. pipette. One loopful of a two-day broth culture of *B. typhosus* (isolated some months previously from a case of typhoid fever) added. For this experiment the flask was kept stoppered with an indiarubber plug and standing in a basin of water in an outside shed where the other experiments were carried out; the temperature of shed and water is given above for all these experiments.

The numbers of *B. typhosus* present were estimated at once and then at intervals as indicated in Table XII.

1 c.c. of the liquid was added to 9 c.c. sterile water = dilution *A*.
1 c.c. dilution *A* added to 99 c.c. = dilution *B*.
1 c.c. dilution *B* added to 99 c.c. = dilution *C* &c.

1·0 and 0·1 c.c. of each dilution added to broth tubes which were subsequently examined for *B. typhosus*. The organism isolated in the greatest dilution for the last examination made, was fully worked out and identified: the others only partially.

Exp. 2. Exactly similar to Exp. 1, except that the mud was rather less liquid and the enumeration was made by adding two platinum loopfuls of mud each time to 9 c.c. sterile water = dilution *A*. Dilutions *B* and *C*, as for Exp. 1. Both experiments started Sept. 2nd, 1904. For the results of the examinations see Table XII *a*.

The characters of the typhoid bacilli isolated after 50 days (Exp. 1) and 56 days (Exp. 2) respectively were worked out and found to be quite unaltered.

These two experiments demonstrate that the typhoid bacillus will readily live in sterile moist mud. They further show that under the conditions of experiment an increase in numbers took place, followed by a diminution; but at the end of 7—8 weeks there was either about the same number or fewer bacilli present than when the experiment was started. They also show that the characters of the typhoid bacillus were not affected by prolonged sojourning in sterile mud. These results although of interest in themselves do not answer the essential question propounded.

Group II. B. typhosus in polluted tidal mud (not sterilized).

This group comprises four separate experiments. For all four experiments large numbers of typhoid bacilli were added to fresh highly polluted tidal river mud in sterile flasks of 300—400 c.c. capacity. The flask was filled with fresh sea water and the mud and sea water thoroughly mixed. Fresh sea water was added twice a week, the procedure being in each case to pour off the stale sea water, take samples of the mud if required, add the fresh sea water, and mix up thoroughly. The flasks were plugged loosely with cotton-wool and kept in the outside shed. For temperatures of the shed see above.

To demonstrate the typhoid bacillus several separate platinum loopfuls of the mud were brushed over series of Drigalski and Conradi plates. All typhoid like colonies were subcultivated into broth and incubated at 37°C. These broth cultivations were examined next day and all those which showed uniform turbidity of the broth and in hanging drop, a possible morphology, and considerable motility were further examined. The others were at once excluded.

The further steps employed were to grow these organisms in lactose peptone water (in Durham's tubes) for acid and gas production, in litmus milk for at least 2 weeks and to test the 24 hours' broth culture in 1 : 1000 dilution with a powerful antityphoid serum (time allowed 2 hours) which readily agglutinated the race of *B. typhosus* used for these experiments in this and higher dilutions. The further tests employed for identification were growth in peptone water for indol production, in

glucose neutral-red agar shake cultures, and on gelatin slope cultures for slow growth, typical appearance and non-liquefaction. The typhoid bacilli last isolated were further stained by Gram's method, grown in mannite peptone water and their gelatin surface colonies examined, while the reactions with the antityphoid serum were repeated.

The organism isolated had to conform in all its characters to that of the typhoid bacillus used for the experiments, before it was accepted as that organism.

Exp. 1. Started Sept. 16th, 1904. 2 c.c. of emulsion in sea water of *B. typhosus* agar slope culture, added.

1*st examination* Sept. 16th. Typhoid bacilli colonies abundant in one loopful of the mud, brushed over a Drigalski and Conradi plate.

2*nd examination* Sept. 30th. *B. typhosus* fairly readily isolated.

3*rd* ,, Oct. 7th. *B. typhosus* not isolated.

4*th* ,, Oct. 11th. ,, ,, ,,

Result. Typhoid bacilli fairly easily isolated after two weeks in the mud, but could not be found after three weeks.

Exp. 2. Started Sept. 16th, 1904. 1·5 c.c. of emulsion in sea water of *B. typhosus* agar slope culture, added.

1*st examination* Sept. 16th. *B. typhosus* easily isolated from 1 loopful of mud.

2*nd* ,, Sept. 22nd. ,, ,, ,, ,, 1 ,, ,,

3*rd* ,, Sept. 30th. *B. typhosus* fairly readily isolated.

4*th* ,, Oct. 7th. *B. typhosus* not isolated.

5*th* ,, Oct. 14th. ,, ,, ,,

Result. Identical with Experiment 1.

Exp. 3. Started Oct. 21st, 1904. Here part of the same mud as that of Tank III was used.

For this experiment an attempt was made not merely to see if the typhoid bacillus was present but to as far as possible estimate its numerical presence. At the same time the number of *B. coli* and streptococci were also estimated every week exactly in the same way as for the tank experiments. The results of the *B. coli* and streptococci enumerations are given in Table XIII.

To estimate the number of *B. typhosus* not only were loopfuls of the mud brushed over Drigalski and Conradi plates, but the MacConkey tubes each containing 0·1, 0·01, etc. grm. of mud were also brushed over similar plates after 1–2 days' incubation at 37° C. A considerable part of an agar slope culture emulsified in fresh sea water was added to the mud (about 100 grms.) so that the infection was a massive one.

1*st examination* Oct. 21st. Here the colonies from one loopful of mud appeared all blue, and supposing they were all *B. typhosus* I only subcultivated one. On working out it was found to be not *B. typhosus*, but an organism closely simulating it. Its most characteristic feature was the marked alkalinity it produced in litmus milk after 8–9 days' growth, for the first few days acid being produced. It did not ferment glucose like *B. enteritidis* however. *B. typhosus* was therefore not isolated.

2nd examination Oct. 29th. *B. typhosus* isolated from 0·00001 grm. as well as from one loopful of mud.

3rd examination Nov. 5th. *B. typhosus* isolated from 0·00001 grm. of mud.

4th examination Nov. 11th. *B. typhosus* isolated from 1 loopful of mud, but not found in any of the dilutions.

5th examination Nov. 18th. *B. typhosus* isolated from 1 loopful of mud but not found in any of the dilutions.

6th examination Nov. 25th (after 35 days). Quite similar to Nov 18th, *i.e.* isolated from 1 loopful of mud.

7th examination Dec. 2nd. *B. typhosus* not isolated.

8th „ Dec. 9th. „ „ „

Result. In this experiment the typhoid bacillus was isolated after 35 days in mud, but could not be found a week later.

When the typhoid bacillus was found *B. coli* were still present in 0·01 grm. (see Table XIII), but streptococci could not be found in 0·1 grm.

The infection with typhoid bacilli was a very gross one however, and even after 2 weeks in the mud the bacillus was isolated in 0·00001 grm. of mud: *i.e.* there were then presumably at least 100,000 typhoid bacilli per gramme of mud, numbers at least equal to the number of *B. coli* present.

Exp. 4. Started Oct. 21st, 1904. The same mud as Exp. 3, and inoculated with approximately an equal amount of agar emulsion. The detection of the bacillus alone was however attempted.

1st examination Oct. 29th (after 8 days). *B. typhosus* easily isolated from 1 loop of mud.

2nd examination Nov. 5th (after 15 days). *B. typhosus* fairly easily isolated.

3rd „ Nov. 11th (after 21 days). *B. typhosus* not isolated.

4th „ Nov. 15th. *B. typhosus* not isolated.

5th „ Nov. 16th. „ „ „

Result. *B. typhosus* fairly easily isolated after 15 days, but could not be found after a longer interval.

Owing to the enormous difficulties inherent to the isolation of the typhoid bacillus from bacteriologically complex substances such as highly polluted tidal mud, it would be rash to draw sweeping deductions from negative results, but from the above it seems justifiable to infer that typhoid bacilli can survive in polluted muds for at least 2 weeks, and this fairly readily, but that after about 2 weeks they very rapidly decrease, although they may, and probably do, persist under favourable conditions for some time longer but in vastly diminished numbers. Experiment (3) seems to definitely show that they may survive for at least 5 weeks.

The fact that the typhoid bacilli may have been present in the mud but not isolated owing to the inherent difficulties of such isolation is probably entirely compensated for by the very massive infection practised, and when the rate of decrease of *B. coli* and streptococci in the

tank muds is considered it seems to me justifiable to assume that if *B. coli* are present in less than 0·01 gramme and streptococci only found in 0·1 gramme or less, the risk of the typhoid bacillus being present, and consequently of specific oyster pollution, can be considered a negligible one.

Group III. B. typhosus + B. coli in sterile mud.

In view of the difficulty of, with certainty, isolating the typhoid bacillus from polluted tidal mud, it occurred to me that if it could be shown that *B. coli* readily killed out *B. typhosus* in sterile mud, to which both had been added, then the problem of the possibility of excluding the presence of the typhoid bacillus from an estimation of the number of *B. coli*, might be answered in another way.

Two separate experiments were carried out from this point of view.

Exp. 1. Polluted tidal mud sterilized in bottle with cotton-wool plug, Oct. 27th. 1 loopful added of an agar slope emulsion in 5 c.c. of sterile water of a typical *B. coli* isolated from mud. Also 1 c.c. of a sterile water emulsion of *B. typhosus* from agar slope. The mud which was quite moist was well mixed.

Examinations made by weighing 1 grm. into 9 c.c. of sterile water = dilution *A*. Dilutions *B*, *C*, *D*, etc. from this as above described.

The number of *B. coli* was determined by adding 1·0 and 0·1 c.c. of each dilution to MacConkey tubes for gas and acid.

The number of *B. typhosus* was ascertained by plating some of these dilutions and also by brushing directly 1 drop (approximately $\frac{1}{30}$ c.c.) of each dilution over a plate, Drigalski and Conradi plates being used throughout. Care was taken to show that only these two organisms were present throughout the experiment.

The results are shown in Table XIV.

Exp. 2. Started Oct. 29th. Rather over 60 grms. of moist mud sterilized in flask with cotton-wool plug.

1 c.c. of *B. typhosus* agar emulsion in sterile tap-water added. The *B. coli* was not added until Nov. 2nd, to give the typhoid bacilli a start, when 0·1 c.c. of a gelatin slope emulsion in tap-water was added. Otherwise the experiment was quite similar to Exp. 1. Both flasks kept in the same shed as the other muds. The results are shown in Table XV.

In both experiments the typhoid bacillus was originally present in larger numbers than the colon bacillus.

Both experiments show that the two bacilli can maintain themselves side by side, and in considerable numbers, in tidal mud for some time—apparently 3–4 weeks under the conditions of the experiments. After that period the typhoid bacilli find the conditions unsuitable and

apparently rapidly die out, or at least are present in vastly diminished numbers. On the other hand the *B. coli* were in both instances present in larger numbers at the end of the experiment than when the experiments were started.

Comparing these results with those obtained with *B. typhosus* alone in sterile mud, it is evident that the colon bacillus exerts after a while a prejudicial influence on the growth of the typhoid bacillus in tidal mud. This is what might be expected, although I was not prepared to find that it was not exerted until after several weeks.

Group IV. Two further experiments were carried out—one with streptococci, the other with *B. coli* in sterile mud.

Exp. 1. A streptococcus isolated from mud was mixed with sterile mud and the number estimated every week. Flask kept in shed. Started Oct. 25th. Streptococci when the experiment was started were more numerous than 10, but less than 100 per grm.

Nov. 1st (after 1 week). Streptococci present in 0·01 grm. (*i.e.* more numerous than 100 per grm.) but greater dilutions not examined.

Nov. 7th (after 13 days). More numerous than 100, less than 1000 per grm.

Nov. 17th (after 23 days). ,, ,, ,, ,, ,, ,, ,, ,, ,,

Nov. 29th (after 35 days). Not found in 0·1 grm. or less.

Dec. 5th (after 41 days). Absent in 1 grm. or less.

In this experiment the streptococcus maintained itself, with a slight increase, up to 3 weeks, but then, or soon after, rapidly died out.

Exp. 2. A typical *B. coli* isolated from mud mixed with sterile mud in flask, and number present estimated after definite intervals. Started Oct. 25th, 1904. *B. coli* numbered more than 10,000, less than 100,000 per grm.

Nov. 8th (after 14 days). *B. coli* numbered more than 10,000, less than 100,000 per grm.

Nov. 23rd (after 29 days). *B. coli* numbered more than 1000, less than 10,000 per grm.

Nov. 29th (after 35 days). ,, ,, ,, ,, 10 ,, ,, 100 ,,

Dec. 6th (after 42 days). ,, ,, ,, ,, 100 ,, ,, 1000 ,,

In this particular experiment a distinct diminution in numbers was obtained, but the bacillus could maintain itself for at least 6 weeks, although after 4 weeks a marked diminution took place.

It may further be mentioned that the *B. coli* isolated from these sterile muds were fully worked out on several occasions after being in sterile mud for 5 or 6 weeks, and were found to have retained their characters unaltered.

None of my experiments bear out the idea that *B. coli* in mud, sterilized or unsterilized, alter some of their characters.

Conclusions.

(1) That mud samples yield more reliable bacteriological evidence of the degree of contamination of a tidal river than either water or oyster samples.

(2) Oyster and water samples only indicate immediate and actually present pollution. Mud samples show evidences of past contamination for at least several weeks, and almost certainly for all the time that specific (typhoid bacilli) contamination is possible.

(3) Muds which show high relative purity are safe for oysters.

(4) Standards of number of *B. coli,* streptococci, etc. if broad, can be set up and will serve as a useful classification of the degrees of purity of a tidal river, and will aid inspection and possibly be largely able to take its place.

I am not prepared at present to give a numerical standard, or from the above experiments to affirm that the same standard is applicable to all rivers.

(5) No evidence was obtained that either *B. typhosus* or *B. coli* alter their characters in tidal mud.

(6) Typhoid bacilli can survive fairly readily for 2 weeks in tidal mud, but after that period their numbers, as a rule, rapidly decline.

TABLE I.

No. in Series	Date of Collection	Source of the sample	Ascertained presence of *B. coli* in amounts of wet mud indicated — Grammes 0·1	0·01	0·001	0·0001	0·00001	0·000001	Do. for Streptococci — Grammes 0·1	0·01	0·001	0·0001	0·00001	Estimated No. per grm. — *B. coli*	Streptococci
2	Jan. 22, '04	Just below the town C and above the sewage outfall	+	+	+	+	+	+	+	+	+	+	−	over 1,000,000	10,000
10	Feb. 24, '04	River above the town C	+	+	−	−	−	−	+	+	+	−	−	100	1,000
14	March 15, '04	About 20 yards below No. 2	+	+	+	+	+	−	+	+	+	−	−	100,000	1,000
15	,, ,,	100 yards above sewage outfall	+	+	+	+	−	−	+	+	+	+	−	10,000	10,000
16	,, ,,	100 yards below sewage outfall. Left bank	+	+	+	+	−	−	+	+	+	−	−	,,	1,000
17	,, ,,	100 yards below sewage outfall. Right bank (same side as outfall)	+	+	+	+	−	−	+	+	+	+	−	,,	10,000
23	Aug. 30, '04	About ¾ mile below sewage outfall. Left bank	+	+	+	+	−	−	+	+	−	−	−	,,	100
24	,, ,,	About 100 yards above No. 23	+	+	+	+	−	−	+	+		−	−	,,	100
25	,, ,,	About 100 yards above No. 24	+	+	+	+	−	−	+	+	−	−	−	,,	100
26	,, ,,	¼ mile below sewage outfall. Left bank	+	+	+	+	+	−	+	+	+	−	−	100,000	1,000
27	Sept. 12, '04	100 yards below sewage outfall. Right bank	+	+	+	+	+	−	+	+	+	−	−	,,	1,000

TABLE II.

No. in Series	Date of Collection	Source of the sample	*B. coli* 0·1	0·01	0·001	0·0001	0·00001	0·000001	Streptococci 0·1	0·01	0·001	0·0001	0·00001	*B. coli* per grm.	Streptococci per grm.
7	Feb. 2, '04	Main channel ¼ mile below township W	+	+	+	−	−	−	+	+	−	−	−	1,000	100
18	June 13, '04	40 yards below where largest W drains enter the river	+	+	+	+	−	−	+	+	+	−	−	10,000	1,000
20	Aug. 30, '04	About ⅛ mile below township W	+	+	+	+	−	−	+	+	−	−	−	,,	100
21	,, ,,	¼ mile below township W	+	+	+	−	−	−	+	+	−	−	−	1,000	100
22	,, ,,	Between R and W	+	+	+	+	−	−	+	−	−	−	−	10,000	10

TABLE III.

No. in Series	Date of Collection	Source of the sample	Presence of *B. coli* in amounts indicated — Grammes 0·1	0·01	0·001	0·0001	0·00001	0·000001	Do. for Streptococci — Grammes 0·1	0·01	0·001	0·0001	0·00001	Estimated No. per grm. *B. coli*	Strepto-cocci
5	Feb. 2, '04	Main channel. ½ mile above creek G. Right bank	+	+	–	–	–	–	+	–	–	–	–	100	10
6	,, ,,	Main channel. Just before creek B. Left bank	+	+	–	–	–	–	+	–	–	–	–	100	10
13	Feb. 26, '04	Main channel between creeks G and P	+	+	–	–	–	–	+	–	–	–	–	100	10
28	Oct. 10, '04	Main channel. Left bank. Just above mouth of creek B. About 200 yards above sewage outfall of town B	+	+	–	–	–	–	–	–	–	–	–	100	less than 10
29	,, ,,	Main channel. Left bank. 200 yards above last sample	+	+	–	–	–	–	–	–	–	–	–	100	,,

TABLE IV.

No. in Series	Date of Collection	Source of the sample	Presence of *B. coli* in amounts indicated — Grammes 0·1	0·01	0·001	0·0001	0·00001	0·000001	Do. for Streptococci — Grammes 0·1	0·01	0·001	0·0001	0·00001	Estimated No. per grm. *B. coli*	Strepto-cocci
1	Dec. 31, '03	Creek G near mouth	+	–	–	–	–	–	–	–	–			10	less than 10
3	Feb. 2, '04	Creek P, ⅓ mile up from mouth	+	–	–	–			+	–	–			10	10
4	,, ,,	Creek P, 50 yards from mouth	+	–	–	–			–	–	–			10	less than 10
8	,, ,,	Creek P, ⅓ mile up from mouth	+	–	–	–			–	–	–			10	,,
9	,, ,,	Creek G, about 200 yards up	+	+	–	–	–	–	+	–	–			100	10
11	Feb. 26, '04	Creek P, ¾ mile up	+	–	–	–			+	–	–			10	10
12	,, ,,	Creek G, ¾ mile up	+	–	–	–			–	–	–			10	less than 10
19	June 29, '04	Creek P, rather less than ¼ mile from mouth	–	–	–	–			–	–	–			less than 10	less than 10

TABLE V.

No. in Series	Date of Collection	Source of the sample	Presence of *B. coli* in amounts indicated — Grammes 0·1	0·01	0·001	0·0001	0·00001	0·000001	Do. for Streptococci — Grammes 0·1	0·01	0·001	0·0001	0·00001	Estimated No. per grm. — *B. coli*	Strepto-cocci
30	Oct. 10, '04	Creek B. About ½ mile up the creek from the ferry. Right bank	+	–	–	–	–	–	–	–	–	–	–	10	less than 10
31	,, ,,	Creek B. About 250 yards nearer the ferry than last sample. Right bank	–	–	–	–	–	–	–	–	–	–	–	less than 10	,,
32	,, ,,	Creek B. About 200 yards nearer mouth than last sample. Right bank	+	–	–	–	–	–	–	+	–	–	–	10	?
33	,, ,,	Creek B. About 50 yards up the creek from the ferry. Right bank	+	+	+	+	–	–	+	+	–	–	–	10,000	100
34	,, ,,	Creek B. Left bank. Near mouth and seaward side of the ferry.	+	–	–	–	–	–	+	+	–	–	–	10	100

TABLE VI.

No. in Series	Date of Collection	Source of the sample	*B. coli* 0·1	0·01	0·001	0·0001	0·00001	0·000001	Streptococci 0·1	0·01	0·001	0·0001	0·00001	*B. coli*	Strepto-cocci
a	June 29, '04	Creek P. ⅓ mile up from mouth. Nearly low water	–	–	–	–				–				less than 10	less than 10
b	,, ,,	Creek P. 100 yards higher up than *a*. Collected at same time	+	–	–	–			–	–	–			10	,,
c	,, ,,	Creek P. 100 yards higher up than *b*. Collected at same time	–	–	–	–			–	–	–			less than 10	,,
d	,, ,,	Creek P. Same place as *a* except that at ½ flood tide, *i.e.* higher level	–	–	–	–			–	–	–			,,	,,
e	,, ,,	Creek P. Same as *b*, but at ½ flood tide	–		–	–			–	–	–			,,	,,
f	,, ,,	Creek P. Same as *c*, but at ½ flood tide	–	–	–	–			–	–	–			,,	,,

TABLE VIII. *Characters of B. coli isolated.*

Reference number	Source	Liquefaction of Gelatine	Gelatine Slope: 2 days	Gelatine Slope: 2 weeks	Glucose Fermentation	Lactose Fermentation	Neutral red Reaction	Litmus Milk: Permanent acidity 2 weeks	Litmus Milk: Coagulation	Indol production	Morphology	Motility	Saccharose Fermentation	
1	Mud 1	–	Bluish translucent growth	unchanged	+	+	+	+	+ (3 days)	+	Short b. rounded ends	+		
2	,, 2	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	–	–	
3	,, 3	–	Transl. growth, but whiter than quite typical	,,	+	+	+	+	+ (24 hrs.)	+	,,	–	+	
4	,, 4	–	Bl. transl. growth	,,	+	+	+	+	+ (2 days)	+	,,	–	–	
5	,, 5	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–	–	
6	,, 6	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	+	–	
7	,, 7 (0·001 grm.)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–	–	
8	,, 8 (0·1 grm.)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	+	–	
9	,, 9 (0·01 ,,)	–	,,	,,	+	+	–	+	+ (24 hrs.)	+	,,	–	–	
10	,, 10 (0·01 ,,)	–	,,	,,	+	+	+	+	+ (3 days)	+	,,	+	–	
11	,, 11 (0·1 ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
12	,, 12 (0·1 ,,)	–	,,	,,	+	+	+	+	+ (6 days)	+	,,	+		
13	,, 13 (0·01 ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–	+	
14	,, 14 (·00001 grm.)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
15	,, 15 (·0001 grm.)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	–	–	
16	,, 16 (0·0001 grm.)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
17	,, 17 (,, ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
18	,, 18 (,, ,,)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	+	–	
19	,, 20 (,, ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
20	,, 21 (·001 grm.)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
21	,, 22 (·0001 ,,)	–	,,	,,	+	+	+	+	+ (3 days)	+	,,	–		
22	,, 23 (,, ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
23	,, 24 (,, ,,)	–	,,	,,	+	+	+	+	+ (6 days)	–	,,	+		
24	,, 25 (,, ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	+	+	Ferments glucose neutral red shake but slightly at first, and with no neutral red reaction.
25	,, 26 (·00001 grm.)	–	,,	,,	–	+	–	+	+ (2 days)	+	,,	–	+	
26	,, 27 (,, ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
27	,, 28 (·01 ,,)	–	,,	,,	+	+	+	+	+ (3 days)	+	,,	+		
28	,, 29 (,, ,,)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	–		
29	,, 30 (·1 ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
30	,, 32 (0·1 ,,)	–	Less translucent & whiter than typ. not crinkled	,,	+	+	+	+	+ (2 days)	+	,,	–		
31	,, 33 (0·0001 grm.)	–	Bl. transl. growth	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
32	,, 34 (0·1 grm.)		,,	,,	+	+	+	+	+ (2 days)	+	,,	–		

33	Mud *b* (0·1 grm.)	–	Bluish translucent growth	un-changed	+	+	+	+	+ (24 hrs.)	–	short b. rounded ends	–	–	+indol on re-examination 2 months later.
34	,, 13 (0·1 grm.)	–	,,	,,	+	+	+	+	+ (,,)	–	,,	+		Indol repeated after 1 week. Marked indol present.
35	,, 10 (0·01 ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	+		
36	,, 15 (,, ,,)	–	,,	,,	+	+	+	+	+ (3 days)	+	,,	–		
37	,, 15 (,, ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
39	Tank I. mud	–	,,	,,	+	+ slow	partial	+	+ (3 days)	+	,,	+	+	Neutral red agar shake repeated several times with same result.
40	,, ,,	–	,,	,,	+	+	+	+	+ (2 ,,)	+	,,	+		
41	,, ,,	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–	+	
42	,, ,,	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–	–	
43	,, ,,	–	,,	,,	+	+	+	+	+ (,,	–	,,	–		Indol test repeated. Same result.
44	,, ,,	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
45	,, ,,	–	Translucent growth thicker & moister than typical form	,,	+	+	+ delayed	+	+ (2 days)	+	,,	–	–	No starch fermentation.
46	,, ,,	–	Bl. transl. growth	,,	+	+	+	+	+ (,,)	+				
47	,, ,,	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
48	,, ,,	–	,,	,,	+	+	+	+	+ (6 days)	+	,,	–		
49	,, ,,	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
50	Tank II. mud	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
51	,, ,,	–	Thick white growth	,,	+	+ +	+	+	+ (,,)	–	,,	–	+ +	Ferments starch slightly. Also raffinose but not dulcite. *Not B. coli.*
52	,, ,,	–	Bl. transl. growth	,,	+	+	+	+	+ (2 days)	+	,,	–		
53	,, ,,	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
54	,, ,,	–	,,	,,	+	+	+	+	+ (4 days)	+	,,	–		
55	,, ,,	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
56	,, ,,	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	+		
57	,, ,,	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
58	,, ,,	–	Whiter and thicker than typical	,,	+	+	partial only	+	+ (24 hrs.)	+	,,	–		No starch fermentation.
59	,, ,,	–	Bl. transl. growth	,,	+	+	+	+	+ (2 days)	+	,,	–		
60	,, ,,	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
62	Tank III. mud	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
63	,, ,,	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	–		
64	,, ,,	–	,,	,,	+	+	+	+	+ (,,)	+				
65	,, ,,	–	,,	,,	+	+	partial	+	+ (24 hrs.)	–	,,	–		Repeated 1 month later. Indol produced.
66	,, ,,	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
67	,, ,,	–	,,	,,	+	+	+	+	+ (,,)		,,	–		
68	Group II. Exp. (3)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	+		
69	,, ,,	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
70	,, ,,	–	,,	,,	+	+	+	+	+ (3 ,,)	+	,,	–		
71	,, ,,	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
72	,, ,,	–	Whiter and moister than typical	,,	+	+	delayed –	+	+ (,,)	–	,,	–	–	Neutral red positive reaction given after one week, after being kept in the laboratory for 2 weeks.
73	,, ,,	–	Bl. transl. growth	,,	+	+	–	+	+ (,,)	+				
74	,, ,,	–	,,	,,	+	+	+	+	+ (2 days)		,,	+		

TABLE VII.

No. in Series	Date of Examination	Source of the sample	Presence or absence of *B. coli* Grammes						Do. for Streptococci Grammes					Estimated No. per grm.	
			0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	0·00001	*B. coli*	Strepto-cocci
6	Feb. 2, '04	Main channel just before creek B. Left bank	+	+	–	–	–	–	+	–	–	–	–	100	10
6	March 3, '04	Same sample but kept in cupboard in laboratory (cold)	–	–	–	–	–	–	–	–	–	–	–	less than 10	less than 10
13	Feb. 26, '04	Main channel between creeks G and P	+	+	–	–	–	–	+	–	–	–	–	100	10
13	March 4, '04	Same sample but kept in laboratory (cold) for a week	+	–	–	–	–	–	-	–	–	–	–	10	less than 10
10	Feb. 24, '04	River above the town C	+	+	–	–	–	–	+	+	+	–	–	100	1000
10	March 9	Same sample but kept in outside shed for 2 weeks	+	+	–	–	–		+	–	–	–	–	100	10
10	March 26	Same sample but kept in outside shed for 30 days	* –	–	–	–	–		+	–	–	–	–	?	10
15	March 15	100 yards above sewage outfall	+	+	+	+	–	–	+	+	+	+	–	10,000	10,000
15	March 29	Same sample but kept in outside shed for 2 weeks	+	+	–	–	–	–	+	+	–	–		100	100
15	April 5	Same sample but kept in outside shed for 3 weeks	+	+	–	–	–	–	+	+	–	–		100	100

* Organisms not typical *B. coli* isolated.

Table IX. *Tank I. Samples.*

Date of Examination	Presence or absence of *B. coli* Grammes 1·0	0·1	0·01	0·001	0·0001	0·00001	0·000001	Do. for Streptococci Grammes 0·1	0·01	0·001	0·0001	*B. coli* isolated
Aug. 31, '04		+	+	+	+	+	−	+	+	+	−	No. 39†
Sept. 6		+	+	+	−	−	−	+	+	−	−	,, 40
,, 13		+	+	+	−	−	−	−	−	−	−	,, 41
,, 21		+	+	−	−			− *	−	−	−	,, 42
,, 27		+	+	−	−			− *	−			,, 43
Oct. 4		+	+	−	−			− *	−			,, 44
,, 11		+	−	−	−			− *	−			,, 45
,, 18		−	−	−	--			− *				—
,, 25		−	−					−				—
Nov. 1	+	−						*				No. 46
,, 8	+	−						*				,, 47
,, 15	−	−										—
,, 22	+											No. 48
,, 29	+											,, 49

* Streptococci also absent in 1 grm. of the mud.
† These numbers refer to the reference number Table VIII.

Table X. *Tank II. Samples.*

Date of Examination	Presence or absence of *B. coli* Grammes 1·0	0·1	0·01	0·001	0·0001	0·00001	0·000001	Do. of Streptococci Grammes 1·0	0·1	0·01	0·001	0·0001	*B. coli* isolated
Sept. 12		+	+	+	+	+	−		+	+	+	−	No. 50
,, 21		+	+	+	+	−	−		+	+	+	−	,, 51*
,, 27		+	+	+	−	−	−		+	+	−	−	,, 52
Oct. 4		+	+	+	+	−	−		+	−	−	−	,, 53
,, 11		+	+	+	−	−	−	− (0·5 g.)	−	−	−	−	,, 54
,, 18		+	+	+	--	-	−	−	−	−			,, 55
,, 25		+	+	−	−			−	−	−			,, 56
Nov. 1		+	−	−	−			−					,, 57
,, 8		+	−	−	−			−					,, 58
,, 15		+	−	−									,, 59
,, 22		−	−										—
,, 29	+	−	−										,, 60
Dec. 6	−	−											—
,, 13	−												
,, 15	−												

* On working out not a true *B. coli*.

Table XI. *Tank III. Samples.*

Date of Examination	Presence or absence of *B. coli* Grammes 1·0	0·1	0·01	0·001	0·0001	0·00001	0·000001	Do. for Streptococci Grammes 0·1	0·01	0·001	0·0001	*B. coli* isolated
Oct. 21		+	+	+	+	+	–	+	+	+	–	No. 62
Nov. 1		+	+	+	+	–	–	+	+	–	–	,, 63
,, 15		+	+	+	+	–	–	+	–	–		,, 64
,, 22		+	+	–	–	–	–	+	+			,, 65
,, 29		+	+	–	–			+	–			,, 66
Dec. 6		–	–					+ (& in 1 g.)	–			—
,, 13*		+	–					– (absent in 1 grm.)	–			,, 67

* Mud again examined Dec. 15th for Streptococci. *Nil* found in 2 grms.

Table XII. *B. Typhosus in sterile mud.*

Experiment (1).

Date of Examination	Interval since experiment started	1·0 A	0·1 A	1·0 B	0·1 B	1·0 C	0·1 C
		c.c. liquid mud					
		0·1	0·01	0·001	0·0001	0·00001	0·000001
Sept. 2	—	+	+	+	+	–	–
,, 9	7 days	+	+	+	+	+	–
,, 27	25 ,,	+	+	+	+	–	–
Oct. 7	35 ,,	+	+	+	+	–	–
,, 22	50 ,,	+	+	+	–	–	–

TABLE XII *a*. *B. Typhosus in sterile mud.*

Experiment (2).

Date of Examination	Interval since experiment started	1·0 A	0·1 A	1·0 B	0·1 B	1·0 C	0·1 C
Sept. 2	—	+	+	−	−	−	−
,, 17	15 days	+	+	+	+	*	
,, 30	28 ,,	+	+	+	+	*	
Oct. 14	42 ,,	+	−	+	−	−	−
,, 28	56 ,,	+	+	−	−	−	−

* Not examined in these dilutions.

TABLE XIII. *Group II., Experiment* (3).

Date of Examination	Presence or absence of *B. coli* Grammes						Do. for Streptococci Grammes				*B. coli* isolated
	0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	
Oct. 29	+	+	+	+	+	−	+	+	+	−	No. 68
Nov. 5	+	+	+	+	−	−	+	+	−	−	,, 69
,, 11	+	+	−	−	−	−	+	−	−	−	,, 70
,, 18	+	+	+	−	−	−	+	+			,, 71
,, 25	+	+	−	−	−	−	−	−			,, 72
Dec. 2	+	−	−	−			−	−			,, 73
,, 9	+	+	−	−			−*				,, 74

* Streptococci also absent in 0·5 grm. of the mud.

TABLE XIV. *Group III., Experiment* (1).

Date of Examination	Interval since experiment started	Presence or absence of *B. typhosus*						Do. for *B. coli*				
		2 loopfuls of mud	$\frac{1}{30}$ c.c. A	$\frac{1}{10}$ c.c. B	$\frac{1}{30}$ c.c. B	$\frac{1}{10}$ c.c. C	$\frac{1}{30}$ c.c. C	1·0 c.c. B	$\frac{1}{10}$ c.c. B	1·0 c.c. C	$\frac{1}{10}$ c.c. C	$\frac{1}{30}$ c.c. C
Oct. 27	–			+	+	+		+	+		–	
Nov. 1	5 days			+	+	+	–	+	+	+	–	–
,, 15	19 ,,		+		+		+	+	+	+	–	
,, 22	26 ,,		+		+		+	+	+	+	+	–
Dec. 5	39 ,,		–		–		–	+	+	+	+	+ (8 colonies)
,, 9	43 ,,	–										

TABLE XV. *Group III., Experiment* (2).

Date of Examination	Interval since *B. coli* added	Presence or absence of *B. typhosus*						Do. for *B. coli*						
		2 loopfuls of mud	$\frac{1}{30}$ c.c. A	$\frac{1}{10}$ c.c. B	$\frac{1}{30}$ c.c. B	$\frac{1}{10}$ c.c. C	$\frac{1}{30}$ c.c. C	1·0 c.c. B	$\frac{1}{10}$ c.c. B	1 c.c. C	$\frac{1}{10}$ c.c. C	$\frac{1}{30}$ c.c. C	1 c.c. D	$\frac{1}{10}$ c.c. D
Oct. 31	–			+	+	+								
Nov. 3	1 day							+	+	+	–			
,, 11	9 days				+	–	–	+	+	+	+	–		
,, 17	15 ,,		+	+	+		–	+	+	+	+	+	+	
,, 24	22 ,,		+		+		–	+	+	+	+	+	+	–
Dec. 6	34 ,,		–		–		–	+	+	+	+	+	–	–
,, 12	40 ,,	–												

AN IMPROVED METHOD OF CALCULATING BIRTH-RATES.

By ARTHUR NEWSHOLME, M.D.,
and T. H. C. STEVENSON, M.D.

In the ten years 1841–50 the birth-rate of England and Wales averaged 32·6 per 1000 of population. It reached its maximum (36·3) in 1876, since which year it has steadily declined to 28·5 in 1901 and 1902, and to 27·9 in 1904.

In Scotland the highest recorded birth-rate was 35·6 in 1876, the same birth-rate being also recorded in 1860 and 1864. The lowest was 29·2 in 1902.

The highest recorded birth-rate in Ireland was 28·0 in 1871, the lowest 22·7 in 1900 and in 1901.

All parts of the United Kingdom, like many other civilized countries, show a marked and continuing decline of the birth-rate, the decline having been exceptionally great in France and in the United States.

The question arises to what extent is this decline, and it may be added the difference in the crude birth-rate of the three parts of the United Kingdom, due to a true change or difference in the fertility of married women; and to what extent to other changes in these communities? These are diminution in the proportion borne by the population at child-bearing ages to the total population, diminution in the proportion borne by the married to the total population at these ages, and postponement of marriages into the later ages of child-bearing, which may be taken as approximately 15 to 45.

It is proposed,

(*a*) to indicate the fallacies underlying the ordinary method of statement of birth-rates:

(*b*) to describe an accurate method of stating the birth-rate: and

(*c*) to discuss results obtained by employing this accurate method. (This will be done in a subsequent paper.)

(*a*) *Fallacies of ordinary method of stating the birth-rate.*

The birth-rate under present conditions of life varies in accordance with the number of men and women of child-bearing ages in the community. There is no evidence that any change in the virility of men or in the potential fertility of women has occurred in the thirty or forty years for which fairly accurate birth-statistics are available: and it is therefore unnecessary in this connection to discuss Herbert Spencer's dictum that "the ability to maintain individual life and the ability to multiply vary inversely[1]."

The birth-rate is usually reckoned as a rate per 1000 of the population living at all ages in the middle of the year. This is the *crude birth-rate*. It is an accurate measure of the relative fertility of two communities only when the number of married women and the ages of the married women in each of these communities in every 1000 of the total population are identical. In such a case it would be a correct statement of fertility, unless illegitimate births were so large a proportion of the total births as to introduce a serious disturbing factor into the problem.

Nor is a statement of the number of births per 1000 total women aged 15–45 an accurate measure of fertility; for in one community a large proportion of these women may be unmarried and their birth-rate be a negligible amount; while in another there may be very few unmarried women at these ages.

The statement of the number of legitimate births per 1000 of the number of married women aged 15—45 constitutes a much more accurate measure of fertility which has been seldom employed.

The following example[2] compares the effect of the application of the above three methods of stating the birth-rate in a concrete instance.

Legitimate Birth-rates in Kensington and Whitechapel in 1891.

	A. Birth-rate per 1000 inhabitants	B. Birth-rate per 1000 women aged 15—45	C. Birth-rate per 1000 married women aged 15—45
Kensington	21·8	61·6	215·4
Whitechapel	39·9	172·1	328·3
Percentage excess of birth-rate in Whitechapel over that in Kensington	83·0 %	179 %	53 %

[1] *A Theory of Population deduced from the General Law of Animal Fertility.* By H. Spencer, 1872.

[2] From Newsholme's *Elements of Vital Statistics*, p. 72, 3rd edit.

The figures in column C of the above table give a much more exact representation than the figures in columns A and B of the true fertility-rate of the two contrasted communities.

A distinction must here be drawn between birth-rate and fertility-rate. The figures in column A show the rates of increase by births in Kensington and Whitechapel in 1891; and from a national and economic standpoint this is the final result which is sought. They do not, however, show how much of the difference in the rates of increase by births in the two districts is owing to differences of fertility, and how much to the arithmetical causes which have been already enumerated. Before considering the true differences in fertility, and even in determining the temporary or permanent importance of changes in the crude birth-rate, these other factors must be eliminated, and we now propose to show how this can be done.

(b) *An accurate method of stating the birth-rate.*

The method of stating fertility-rates given in column C of the preceding table can only be accurately employed in comparing the corresponding figures of two or more communities when (1) of the total wives aged 15–45 the proportions at ages 20–25, 25–30, 30–35 and so on are identical in the communities compared; and when (2) there is an equal proportion in the compared communities of newly married women in each of the different age-groups. In actual communities these conditions are never fulfilled.

The first condition is necessary because of the greater fertility of young than of older married women, as exemplified by the following fertility-rates for Swedish wives in 1891[1].

Swedish Wives 1891.

Ages of Wives	Births annually per 1000 wives
15—20	518
20—25	451
25—30	375
30—35	312
35—40	250
40—45	142

The second condition is involved in the well-known fact that the fertility-rate at any given age is higher among recently married wives

[1] Quoted from a paper on "The Declining Birth-rate in Australia." By W. McLean, *Intercol. Med. Journ. of Australasia*, Mar. 20, 1904.

than among wives longer married, especially in the later years of child-bearing. The figures quoted below from the Tables of Natality published by Kőrösi[1] (based on 46,931 births in Budapest) show the influence of recent marriage in increasing the fertility-rate in a given year of married life as well as the variations in the fertility-rate according to age.

Age in years	The number of children born within a year to every 1000 — Of women newly married	Of all married women
30—34	329	206
35—39	327	147
40—44	214	59

For errors arising from the non-fulfilment of the first condition exact correction can be made by the method to be shortly described; for the non-fulfilment of the second condition no correction appears to be practicable. The first source of error tends to understate the fertility-rate of residential when compared with industrial districts, because in the former the proportion of the total wives aged 15—45 who are at the younger child-bearing ages is smaller than in the latter. The second source of error acts in the opposite direction. The proportion of newly married women in the later age-groups is probably higher in residential districts where women marry comparatively later in life than in industrial districts where women marry at earlier ages, and the fertility-rate at the higher child-bearing ages will therefore be greater in the residential than in the industrial districts. The second possible source of error presupposes that prudential considerations diminishing fertility come into operation more particularly in later married life, which is probably the case, or that the fertility of long married women gradually becomes exhausted even though they are still technically within the child-bearing limit.

The first error is corrected by a method which is analogous to that employed by the Registrar-General in his Annual Summaries in obtaining factors of correction by means of which corrected death-rates are calculated.

The first portion of this method is shown in the following example:

[1] See *Elements of Vital Statistics*, p. 66.

BERKSHIRE, 1901.

Wives aged	No. of wives	Fertility-rate per 100 wives at each age-period. Sweden	Calculated No. of births
15—20	139	51·8	72·002
20—25	2671	45·1	1204·621
25—30	6074	37·5	2277·750
30—35	7305	31·2	2279·160
35—40	7063	25·0	1765·750
40—45	6407	14·2	909·794
	29,659		8509·077

$$\text{Standard Fertility-rate} = \frac{\text{Calculated Births} \times 1000}{\text{No. of wives aged 15—45}} = \frac{8509077}{29659} = 286{\cdot}9.$$

Standard Fertility-rate of England & Wales (1901) similarly calculated = 298·55.

$$\text{Factor of Correction} = \frac{\text{Standard rate of England \& Wales}}{\text{Standard rate of Berkshire}} = \frac{298{\cdot}55}{286{\cdot}9} = 1{\cdot}0406.$$

The standard fertility-rates for Berkshire and England given above give the total fertility of the wives of child-bearing ages in these two communities, on the supposition that the fertility-rates of these two populations were the same at each age-period as obtained in Sweden in 1891, the Swedish population representing a fairly normal population. The standard fertility-rate does not therefore represent any fact, but merely serves as a measure of the favourable or unfavourable constitution of the population of a given community for furnishing a high fertility-rate. If a large proportion of the wives are young, the standard rate is high; if only a comparatively small proportion, it is low. In the above example the wives of Berkshire were somewhat less favourably aged for child-bearing than those of England and Wales as a whole. The Berkshire recorded fertility-rate (*i.e.* the number of legitimate births per 1000 wives aged 15–45) must accordingly be increased in proportion to the difference between the two standard rates, in order to render Berkshire comparable with England and Wales.

As the standard fertility-rate is merely used as a measure of favourable or unfavourable age distribution, and as the same measure is applied to all the populations compared, any convenient fertility-rates may be employed, so long as they correctly represent the differences in fertility between the various age-periods. If in the above example the Swedish rates used were increased or decreased in any given proportion the resulting factor of correction would be unchanged, so long as the relation between the different rates remained unaltered.

McLean in the already mentioned paper employed standard fertility-rates calculated as shown above; and his comparisons are therefore restricted to comparisons of the same community at different times and can only be applied very indirectly to the task of comparing different communities.

By calculating corrected fertility-rates different communities can be made directly comparable. Thus in the example of Berkshire, 1901, taken before,

Calculated number of births (as before) = 8509·077.

Factor of correction = 1·0406.

Recorded fertility-rate $= \dfrac{\text{births} \times 1000}{\text{wives aged } 15\text{—}45}$ in Berkshire in 1902 = 219·7.

Corrected fertility-rate = 219·7 × 1·0406 = 228·6.

Such corrected fertility-rates for different communities are strictly comparable. There are, however, several objections to them. The method of statement is unfamiliar. It is necessary to refer to the census figures relating to wives aged 15–45 for each population before the fertility-rate can be calculated, whereas the total population for each community is accessible without reference to census returns. The most important objection is that the fertility of the population as a whole depends not merely upon the ages of its married women, but also upon their number. For these reasons it is desirable to obtain a corrected birth-rate which gives the corrected number of legitimate births in terms of the entire population, and which will thus be similar to, though more accurate than, the familiar crude birth-rate. Such a birth-rate if truly corrected will include compensation for, 1st, the ages and, 2nd, the number of the wives capable of child-bearing. This compensation could be effected in the example of Berkshire taken before by (1) multiplying its crude birth-rate by the factor 1·0406, which would compensate for the higher average age of the Berkshire wives; and then (2) multiplying this result by another factor $\dfrac{116{\cdot}9}{104{\cdot}6}$ to remove the handicap due to its containing only 104·6 wives aged 15–45 per 1000 of its total population, as compared with 116·9 in England and Wales.

The same result is obtained more easily in one stage by the following method, in which standard birth-rates instead of standard fertility-rates are calculated:

BERKSHIRE, 1901.

Calculated no. of births (as before)		= 8509·077.
Total population at census		= 283,531.
Standard birth-rate	$=\dfrac{8509{\cdot}077 \times 1000}{283531}$	= 30·01.
Similarly standard birth-rate of England and Wales		= 34·91.
Factor of correction	$=\dfrac{34{\cdot}91}{30{\cdot}01}$	= 1·1633.
Recorded legitimate birth-rate of Berkshire in 1902		= 22·78.
Corrected " " " "		= 26·50.

The standard birth-rates take into account both the ages and the relative number of the wives, and the resulting factor therefore corrects for both.

For towns it has been found impracticable to calculate standard birth-rates from the Swedish fertility-rates for quinquennial age-periods, because the English census figures only give ages in decennial age-periods after the age of 25. It has therefore been necessary to use a fertility-rate for the age-period 25–35 derived from the Swedish rates for 25–30 and 30–35, and similarly for 35–45. This was obtained by adding together the calculated births at ages 25–30 and 30–35 in England and Wales, and applying the figure so obtained to the number of wives at ages 25–35, in order to obtain the fertility-rate for the whole period.

$$\frac{\text{Calculated no. of births at ages 25—35} \times 1000}{\text{No. of wives at ages 25—35}} = \text{fertility rate at ages 25—35.}$$

It became a matter of interest to ascertain whether this larger grouping of ages introduces any considerable error. This has been tested for some of the counties in which both methods are practicable, and the following table shows the standard birth-rates according as four or six age-groups are employed.

		Standard birth-rate	
		When six age-groups are employed	When four age-groups are employed
Kent	1901	32·96	32·98
Sussex	"	30·21	30·38
Lancashire	"	36·56	36·56
Durham	"	38·04	37·89
Ireland	1881	23·57	24·06

It is only in the abnormally constituted population of Ireland that any considerable difference exists between the standard birth-rates and resulting factors of correction calculated on the two sets of figures.

In the case of Ireland where in 1901 four-group figures had to be used (the Irish census figures for 1901 not giving the ages of wives in six groups) the resultant error is one of understatement of the required correction, which, as will be seen, is already sufficiently striking. Sussex, a residential county, also with a somewhat high average age of wives, shows (a much smaller) error in the same direction. The same explanation applies in both cases. In the combined group 25–35 the proportion of wives aged 30–35 is greater in Ireland and in Sussex than it is in England and Wales, and the combined fertility-rate for the whole group should be lower. Hence the application of the combined rate deduced from the English figures somewhat overestimates the births which the Irish or Sussex wives in this age-group would furnish. Similarly for the age-groups 35–45. Except in the case of Ireland the difference is so trifling that it may be disregarded. On the other hand Durham, selected for the test on account of the large proportion of young wives in its population, shows, as might have been expected, a difference in the opposite direction. Migration as well as early marriage increases the proportion of young wives in industrial communities and has decreased the number in Ireland.

Factors of correction correcting for the proportionate number of wives aged 15–45 in a given population and taking no account of the age distribution of these wives give birth-rates corresponding to the fertility-rates illustrated in column C of the table on page 176. The following table (column B) compares the factors of correction obtained by this means for a number of English counties in 1901, with the accurate factors obtained as described on page 181 (column A).

The means by which the factors in column B have been obtained are indicated by the following example:

The percentage of married women aged 15—45 in England & Wales in 1901 = 11·69.
" " " " Berkshire " = 10·46.

$$\text{The factor of correction for Berkshire} = \frac{11{\cdot}69}{10{\cdot}46} = 1{\cdot}1176.$$

As might be expected, the difference between the two sets of factors in columns A and B when appreciable points to under-correction by the factors in column B. This arises from the fact that the average age of the wives is low in those counties in which their proportionate numbers are high. Hence factors which are high owing to a small proportion of wives (col. B) need to be still higher to correct for the high average age of the wives (col. A) and *vice versâ*. Thus, contrast Sussex and Durham

in which the two sets of factors differ greatly; while Kent and Lancashire have approximately equal factors by both methods. The explanation is doubtless the same as that advanced for the differences between standard birth-rates for the same counties in the last table; the age distribution of wives in Kent and Lancashire corresponding with that of England and Wales, while the average age of wives is high in Sussex and low in Durham.

Factors of Correction for 1901.

	A. Taking into account the age distribution of wives between 15 and 45	B. Not taking into account the age distribution of wives between 15 and 45
Devonshire ...	1·1072	1·0764
Cumberland ...	1·1602	1·1427
Durham ...	·9177	·9512
Berkshire ...	1·1633	1·1176
Rutland ...	1·3381	1·2476
Kent ...	1·0592	1·0531
Sussex ...	1·1556	1·1197
Lancashire ...	·9546	·9590
Westmorland ...	1·2891	1·2177
Essex ...	·9458	·9450
Surrey ...	1·0848	1·0579
Cambridge ...	1·1382	1·1123
Cornwall ...	1·1656	1·1262
Cheshire ...	1·0268	1·0183
Derby ...	·9684	·9766

The corrections are in every instance in the same direction but the differences in their extent are in many cases so considerable as to render it well worth while to adopt the more complete method.

Summary.

1. The ordinary method of calculating the birth-rate does not distinguish between the influence of fertility and of variations in conditions of the population as to age and marriage.

2. In ascertaining the true meaning of the great reduction of the birth-rate which has occurred in the last 25 years it is necessary to have means for distinguishing between the accidental and the intrinsic causes of change.

3. A step in the right direction is made when the legitimate births are stated in terms of the married women at child-bearing ages, and the illegitimate births in terms of the unmarried women of the same ages.

4. This method fails to correct for the differences of fertility of the various ages comprised in the age-period 15–45.

5. By calculating standard fertility-rates for given populations McLean overcame the above difficulty, and was thus able to compare experiences of a given community at different times with the standard.

6. In this paper it is shown that by continuing the above process and obtaining corrected fertility-rates, the fertility-rates of different communities can be made directly comparable.

7. The inconveniences of this new and unfamiliar method, and the necessity involved in it of calculating the crude as well as the corrected fertility-rate in every instance, indicate the desirability of obtaining a factor for each community which throughout an entire intercensal period can be applied to the crude birth-rate of that community.

8. The desirability of such a factor is increased by the fact that the method of corrected fertility-rates does not take into account the proportion of married women in each population.

9. In this paper a method is described of obtaining factors, which, when applied to the readily available crude birth-rates, correct completely both for the varying proportion of married women in compared populations and for the varying fertility at different periods of married life.

10. The practical bearings of these corrected birth-rates will be discussed in a later paper.

Postscript.—Since the preceding paper was sent to the printer, the 66th Annual Report of the Registrar-General of Births, Deaths etc., in England and Wales (1903) has been published; and this report on p. xvii gives a valuable official confirmation of the desirability of correcting crude birth-rates for variations relating to age and married condition. Such corrections are given for England and Wales as a whole, the method adopted being the same as is shown for Kensington and Whitechapel (1891) on p. 176. It is stated on p. xix that "the disturbing factor of changing constitution of the population is mainly, though not entirely, eliminated by calculating the proportion of births to the number of women living at child-bearing ages." As we have seen, this method does not suffice for complete correction, and it is satisfactory to note that on p. xvii attention is also drawn to the influence of changes in the age of married women in the remark that "among married women (at 15 to 45) the proportion of those at ages under 25 years has continuously decreased."

AN IMPROVED METHOD OF CONSTRUCTING SHORTENED LIFE-TABLES.

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THE following is an addendum to a paper which appeared in the last number of this Journal.

The primary object of the paper was to give working formulæ for the construction of a shortened Life-Table, set forth with sufficient clearness to enable them to be practically used by anyone who understands ordinary arithmetic and the use of logarithms, without any necessary comprehension of the principles of the differential or of the integral calculus, by means of which the formulæ have been deduced.

However, there are probably but few who would undertake the task of constructing a Life-Table without interesting themselves in some degree concerning the mathematical principles involved, and, to those at least who may understand something of the method of "interpolating" terms in any given series by means of "Lagrange's formula," it is easily possible to demonstrate how the special formulæ which have been recommended may be very nearly arrived at by simple processes of interpolation only, and in so doing, those who take the trouble to follow the attempted explanations may be brought nearer to a knowledge of what is meant by the differential and integral calculi.

An attempt was made, by means of Fig. 1 and the references to it in the text of the paper (see pp. 89—90), to show how by interpolation and summation of intermediate ordinates at successively decreasing equal intervals the formula of "integration" to be used may be led up to, or in other words, how the "limit of summation" in the given series may be defined.

The following is an explanation of the method by which the first of the successive approximations to the true formula was obtained.

The first step in the problem is, having given the series $u_{-7\frac{1}{2}}$, $u_{-2\frac{1}{2}}$, $u_{2\frac{1}{2}}$, and $u_{7\frac{1}{2}}$, to interpolate $u_{-1\frac{1}{2}}$, $u_{-\frac{1}{2}}$, $u_{\frac{1}{2}}$, and $u_{1\frac{1}{2}}$.

According to "Lagrange's formula," having given any series of linear quantities (that is, such as are capable of being represented as ordinates of a curve), which may be denoted by the symbols u_a, u_b, u_c, u_d, etc., any other term u_x in the series may be interpolated thus (the special case of only four given quantities is now being considered, but it will be obvious how the formula may be applied to five or more):

$$u_x = \left[\frac{(x-b)(x-c)(x-d)}{(a-b)(a-c)(a-d)}u_a + \frac{(x-a)(x-c)(x-d)}{(b-a)(b-c)(b-d)}u_b \right.$$
$$\left. + \frac{(x-a)(x-b)(x-d)}{(c-a)(c-b)(c-d)}u_c + \frac{(x-a)(x-b)(x-c)}{(d-a)(d-b)(d-c)}u_d\right].$$

By substituting $-7\frac{1}{2}$, $-2\frac{1}{2}$, $2\frac{1}{2}$ and $7\frac{1}{2}$ respectively for a, b, c, and d, and then in succession making $x=-1\frac{1}{2}$, $-\frac{1}{2}$, $\frac{1}{2}$, and $1\frac{1}{2}$, the required values can be readily obtained. (It is necessary to be careful about the signs of the individual factors, thus, $-1\frac{1}{2}-(-2\frac{1}{2})=-1\frac{1}{2}+2\frac{1}{2}=+1$, and $-1\frac{1}{2}-2\frac{1}{2}=-4$, and also about counting the number of $-$ signs in the reduced factors of the numerators and denominators of the coefficients of u_a, u_b, etc., and if the number be odd to make the sign $-$, and if even $+$. It may also be noted that the sum of all the coefficients in the expression for u_x should $=1$.)

It is found that

$$u_{-1\frac{1}{2}} = [-6u_{-7\frac{1}{2}} + 108u_{-2\frac{1}{2}} + 27u_{2\frac{1}{2}} - 4u_{7\frac{1}{2}}] \div 125,$$
$$u_{-\frac{1}{2}} = [-8u_{-7\frac{1}{2}} + 84u_{-2\frac{1}{2}} + 56u_{2\frac{1}{2}} - 7u_{7\frac{1}{2}}] \div 125,$$
$$u_{\frac{1}{2}} = [-7u_{-7\frac{1}{2}} + 56u_{-2\frac{1}{2}} + 84u_{2\frac{1}{2}} - 8u_{7\frac{1}{2}}] \div 125,$$
$$u_{1\frac{1}{2}} = [-4u_{-7\frac{1}{2}} + 27u_{-2\frac{1}{2}} + 108u_{2\frac{1}{2}} - 6u_{7\frac{1}{2}}] \div 125.$$

On taking the sum of these four values $+u_{-2\frac{1}{2}}+u_{2\frac{1}{2}}$, dividing by 6, and reducing to denominator of 24, the result obtained is

$$\frac{12{\cdot}8\,(u_{-2\frac{1}{2}}+u_{2\frac{1}{2}}) - {\cdot}8\,(u_{-7\frac{1}{2}}+u_{7\frac{1}{2}})}{24}.$$

By proceeding similarly the results already given for interpolations at intervals of $\frac{1}{2}$ year and $\frac{1}{4}$ year may be obtained, but it is only necessary to have four successive terms, and therefore, as the first term is already given, only three terms need be interpolated; when this is done the series can be summed by means of a formula.

If the interval be made $\frac{1}{10}$ year the coefficients in the numerator

become 12·98 and $-$ ·98; if $\frac{1}{100}$ year, they become 12·998 and $-$ ·998; if $\frac{1}{1000}$ year, 12·9998 and $-$ ·9998, and so on.

Thus by making the intervals less and less, nearer and nearer approximations are obtained to the limit

$$\frac{13\,(u_{-2\frac{1}{2}} + u_{2\frac{1}{2}}) - (u_{-7\frac{1}{2}} + u_{7\frac{1}{2}})}{24}.$$

(See Note 1.)

Fig. 1 having been intended to illustrate interpolation and *summation*, attention may now be directed to Fig. 2, which was designed to illustrate interpolation and *differencing*. (It may be remarked in passing that the only reason why the curve in one figure has been made convex and in the other concave has been to illustrate the point that some parts of Life-Table curves are convex and others concave.)

For the sake of simplicity let it now be supposed that the given five ordinates in Fig. 2 denoted by the general symbol u_x represent numbers of *population* at age x and upwards.

When five equidistant ordinates are given, separated by n units of interval, their relative distances apart measured from the central ordinate are $-2n$, $-n$, 0, n, and $2n$.

Now it can be shown by means of the differential calculus that the "differential coefficient," or in other words, the "limit of differencing" at the central point 0

$$= \frac{8\,(u_n - u_{-n}) - (u_{2n} - u_{-2n})}{12n}.$$

In the special instance now being considered $n = 5$.

It is now proposed to show how this limit may be approximately defined by simple interpolations.

x of course represents age in years. If x be increased by h, which may represent a number of years, a single year, or a fraction of a year, and if the ordinate u_{x+h} be interpolated, then the number belonging to the age-period x to $x+h$ will be measured by $u_{x+h} - u_x$, and $\frac{u_{x+h} - u_x}{h}$ will be the ratio of *number* to *length of period*, and may be considered to represent approximately the number living at the age which is the central point of the interval x to $x+h$.

Now it is obvious that if we wish to calculate thus the number of those supposed to be living near to the exact age x, the smaller we make h the more accurate will be the result.

The differential calculus measures the ratio $\frac{u_{x+h} - u_x}{h}$ when h vanishes, that is, when $h = 0$, and thus defines the "limit of differencing."

Similar reasoning applies to the ordinate u_{x-h}, and the smaller h is made the more nearly will $\frac{u_x - u_{x-h}}{h}$ approach $\frac{u_{x+h} - u_x}{h}$.

In order to obtain a symmetrical formula we may therefore take

$$\frac{1}{2}\left(\frac{u_{x+h} - u_x}{h} + \frac{u_x - u_{x-h}}{h}\right) = \frac{u_{x+h} - u_{x-h}}{2h}$$

as an approximation to the true "differential coefficient" which will become closer and closer to the true value as we make h smaller and smaller.

As a first approximation we may take $x = 0$ and $h = 1$; we have then to find the value of $\frac{u_1 - u_{-1}}{2}$.

By "Lagrange's formula" having given the series u_{-10}, u_{-5}, u_0, u_5, u_{10},

$$u_1 = [9u_{-10} - 66u_{-5} + 594u_0 + 99u_5 - 11u_{10}] \div 625,$$

$$u_{-1} = [-11u_{-10} + 99u_{-5} + 594u_0 - 66u_5 + 9u_{10}] \div 625,$$

$$\therefore\ \tfrac{1}{2}(u_1 - u_{-1}) = [165(u_5 - u_{-5}) - 20(u_{10} - u_{-10})] \div 1250,$$

and on reduction to denominator of 60 this expression becomes

$$[7{\cdot}92(u_5 - u_{-5}) - {\cdot}96(u_{10} - u_{-10})] \div 60.$$

By making h smaller and proceeding similarly the coefficients of the expressions arrived at are as follows:

when $h = \frac{1}{2}$, 7·98 and − ·99,

when $h = \frac{1}{4}$, 7·995 and − ·9975,

when $h = \frac{1}{10}$, 7·9992 and − ·9996,

when $h = \frac{1}{100}$, 7·999992 and − ·999996.

As we have seen, the limit when $h = 0$ is

$$[8(u_5 - u_{-5}) - (u_{10} - u_{-10})] \div 60.$$

(See Note 2.)

When the given series of u_x values represent the *logarithms* of the numbers of population, at age x and upwards, the logarithm of the "differential coefficient" at age 0 (which is strictly speaking the *ratio* $\frac{u_{x+h} - u_x}{h}$ when $h = 0$, but which may be taken as representing the

number living at the exact age represented relatively by 0 in the given series) is found by the expression

$$u_0 + \log \left[8\left(u_5 - u_{-5}\right) - \left(u_{10} - u_{-10}\right)\right] - 1{\cdot}4159356,$$

where 1·4159356 = the logarithm of "M," the "modulus of the common logarithm," + the logarithm of 60.

In the actual work of calculating $\log p'_x$ values the factors $\frac{1}{M}$ and $\frac{1}{60}$ occur both in the numerator and denominator of the fraction $\frac{2P-d}{2P+d}$ and are cancelled out.

The explanation of the above formula in so far as it is modified in dealing with logarithms instead of numbers can only be given by referring to the "Exponential Theorem" and the "Theory of Logarithms," etc., and it is not proposed to attempt it here. Those who may be unable to verify it may take it upon trust, not merely on the authority of the writer, but as having been deduced by a master of the art of applying abstract mathematical principles to the elucidation of problems relating to vital statistics, Mr A. C. Waters.

Note 1.

This limit may be *exactly* obtained thus.

According to the integral calculus, having given a function of three orders of differences,

$$\int_{-n}^{n} u_x dx = 2nA + \frac{2n^3C}{3} = \frac{n}{3}\left(6A + 2n^2C\right).$$

The given series of u_x values being represented by u_a, u_b, u_c, and u_d, the value of $A = u_0$ may be found from the expression of Lagrange's formula already given on p. 185 by substituting 0 for x.

Thus $$u_0 = \frac{-bcd}{(a-b)(a-c)(a-d)} u_a + \dots.$$

The value of C is expressed as follows.

$$C = \left[\frac{-(b+c+d)}{(a-b)(a-c)(a-d)}\right] u_a + \left[\frac{-(a+c+d)}{(b-a)(b-c)(b-d)}\right] u_b + \left[\frac{-(a+b+d)}{(c-a)(c-b)(c-d)}\right] u_c + \left[\frac{-(a+b+c)}{(d-a)(d-b)(d-c)}\right] u_d.$$

When the given terms of the series are equidistant, and when -3, -1, 1, and 3 are substituted respectively for a, b, c, and d,

$$A = \frac{9\left(u_{-1} + u_1\right) - \left(u_{-3} + u_3\right)}{16}.$$

$$C = \frac{-\left(u_{-1} + u_1\right) + \left(u_{-3} + u_3\right)}{16n^2}.$$

$$\therefore \frac{n}{3}(6A+2n^2C)=\frac{n}{3}\left[\frac{13(u_{-1}+u_1)-(u_{-3}+u_3)}{4}\right],$$

and on dividing this last expression by $2n$ it becomes

$$\frac{13(u_{-1}+u_1)-(u_{-3}+u_3)}{24}.$$

Note 2.

This limit may be *exactly* defined thus.

According to the differential calculus when

$$\phi(x)=A+Bx+Cx^2+\ldots,$$
$$\phi'(x)=B+2Cx+3Dx^2+\ldots,$$
$$\therefore \text{ when } x=0,\ \phi'(0)=B.$$

Having given a series of five linear quantities represented by u_0, u_a, u_b, u_c, and u_d,

$$B=\frac{-bcd}{a(a-b)(a-c)(a-d)}u_a+\frac{-acd}{b(b-a)(b-c)(b-d)}u_b+\frac{-abd}{c(c-a)(c-b)(c-d)}u_c$$
$$+\frac{-abc}{d(d-a)(d-b)(d-c)}u_d-\left(\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}\right)u_0.$$

When the given terms are equidistant and when -2, -1, 1, and 2 are substituted respectively for a, b, c, and d, it is evident that the coefficient of u_0 is zero, and the formula will be found to work out to the value already given, the factor n coming into the denominator because in the expressions for the coefficients of u_a, u_b, etc., there are *three* factors in the numerators and *four* in the denominators.

OBSERVATIONS RELATING TO THE STRUCTURE AND GEOGRAPHICAL DISTRIBUTION OF CERTAIN TRYPANOSOMES.

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THE following notes on trypanosomes which I have observed in certain mammals, birds, and goldfish obtained in the neighbourhood of Elstree in Hertfordshire appear worthy of publication, for the reason that our knowledge regarding the geographical distribution of trypanosomes is far from complete. No description has as yet been given of the trypanosomes found in bats by various observers, and a species encountered by me in the mole is, I believe, new.

I. Mammals.

Trypanosomes of Bats.

Laveran and Mesnil (1904, p. 104) state that trypanosomes have been observed by Dionisi in *Miniopterus schreibersii*, in other bats by Testi (1902) and Sambon (1904) in Italy, and by Durham (1900) in Parà; Donovan in Madras found trypanosomes in *Pteropus medius*. Hitherto no account has been published of the structure of bat trypanosomes[1].

Of eight bats caught here during the summer (June—July, 1904) (*Pipistrellus pipistrellus*) and examined shortly after death three showed trypanosomes in their blood.

[1] During the preparation of this paper for publication an account of trypanosomes occurring in bats in North Africa has been given by Ed. and Ét. Sergent (1905). They describe two forms, a small and a large trypanosome, the latter occurring rarely, and having been seen only in the fresh state. The small forms were found in 10 *Vespertilio kuhli* (Natterer) out of 26 examined, and in 7 *Myotis murinus* (Schreber) out of 35 examined. The large trypanosome was found in 2 *V. kuhli* (26 examined). The observers have named the small form *Trypanosoma nicolleorum* and the large one *T. vespertilionis*.

Numerous trypanosomes were found in the blood of one of the infected bats (a young female); the organisms disappeared from a hanging-drop preparation which was kept at 36° C. overnight. In the second bat a few trypanosomes were seen in the peripheral blood. The animal was etherised and the heart-blood examined but none could then be seen. In the third bat the trypanosomes were abundant in the blood. The skin of this bat was infested with fleas. Careful examination revealed no other external parasites on the skin.

A little of the blood from the last bat was inoculated intraperitoneally into a young white rat but an infection did not follow. Cultivation of the trypanosomes was attempted on rabbit-blood agar, kept at room temperature. At the end of 8 days numerous parasites were seen and one small clump of 2 or 3 members was suggestive of commencing multiplication. Unfortunately the medium became contaminated by a mould and the organisms soon disappeared.

It may be added that a specimen of a larger species of bat (*Pterygistes noctula*) showed no trypanosomes in its blood.

Structure of the Bat Trypanosome.

In a fresh preparation the movements of this trypanosome are very active, and seem to indicate a greater contortion than in the case of the rat trypanosome. It appears, moreover, to be considerably smaller than *Trypanosoma lewisi*. Preparations stained by Leishman's method show that it is in reality a small organism. Measurements were difficult on account of the almost invariably contorted position of the parasite in stained preparations. The length of one, which was in an extended position, was 16 μ (including the flagellum); the flagellum measured 8 μ. The breadth, including the membrane, near the macronucleus, was 1·5 μ.

In stained specimens the appearance of this trypanosome is quite characteristic, the large majority forming a circle or oval on account of the apposition of the anterior and posterior ends. Another distinctive feature lies in the close approximation of the macronucleus to the anterior end. The posterior extremity is pointed. The macro- and micro-nucleus stain deeply, while the protoplasm of the body of the parasite stains uniformly pale-blue—no granules being observable. The general appearance of the stained organism is such as to render its differentiation from the trypanosomes hitherto described a very easy matter.

Trypanosome of the Rabbit.

In a previous paper (1904) I described certain features of a trypanosome found accidentally in the blood of three tame rabbits, the parasite resembling very closely that of the rat. Microscopically it is impossible to distinguish them. Another similarity is found in the fact that the rabbit trypanosome is able to live for at least a month outside the body if kept at a comparatively low temperature.

Further observations have shown that tame rabbits are rarely infected; thus, 230 rabbits have been examined without discovering a single infected animal. Forty wild rabbits have, however, yielded 4 positive results. It may, therefore, be fairly assumed that the wild variety is more frequently infected than the tame, exactly as in the case of the rat. To ensure that the parasites should not be overlooked, in eleven instances 10 c.c. of blood were defibrinated and centrifugalized, but the results were again negative.

So far I have not been successful in transmitting the infection from the wild variety to tame rabbits by inoculation; and attempts to infect white rats have also been unsuccessful.

A culture in rabbit-blood agar of trypanosomes from one of the wild rabbits has recently been obtained. After being kept for 16 days at room temperature a hanging-drop preparation was made and showed dividing forms, though not in great numbers. The largest number seen in any one field under a $\frac{1}{12}$ oil immersion lens was 12, arranged in groups of 5, 4 and 3 members; whereas in the original blood many fields had to be examined before one was observed. The forms seen were similar to those in cultures of *Trypanosoma lewisi.*

Trypanosoma lewisi.

Trypanosomes occur in wild rats in this locality with considerable frequency. No exact data are available but the proportion infected may be stated roughly as 30 %. In a lot of 6 rats captured in one place in the middle of December 1 full-grown and 4 young rats were found to be infected. The blood of 2 of the young rats contained numerous dividing forms, including rosettes consisting of small trypanosomes—one group being composed of 10 individuals. It may be noted that this stage is very rarely observed in wild rats; the only observers of it hitherto being Sivori and Lecler. In specimens stained by Leishman's method numerous forms arising from division were seen

similar to those observed in an artificially produced infection. Special attention was paid to the character of the rosettes in stained preparations in view of the recently published observations of MacNeal (1904) on the multiplication forms. MacNeal contends that the grouping by Laveran and Mesnil of the methods of division cannot be accepted. The latter observers classify the dividing forms into two groups, the 1st including those arising from unequal longitudinal division, the 2nd consisting of forms in which there is "a simultaneous division into several coequal daughter-cells, no mother-cell being distinguishable." The forms which I have observed in the specimens of the natural infection undoubtedly support the views held by MacNeal. In every rosette the flagellum of the parent cell can be easily distinguished, while on the other hand on no occasion have rosettes been seen which resemble the figures given by Laveran and Mesnil. The anomalous forms referred to by MacNeal, in which a long, slender extension of the posterior extremity occurs, were also noted. The significance of this does not appear to be known.

Mole (Talpa europaea).

The blood of a mole was examined a few hours after death. There were a very few trypanosomes present, which appeared to be similar in size and form to the rat trypanosome. The blood was pipetted into rabbit-blood agar, and kept at room temperature. 24 hours later one trypanosome with extremely active movements was seen in a hanging drop. Cultivation proved unsuccessful owing to contamination. Films of the blood were stained, but stained organisms could not be found. 3 moles captured in the month of January were examined with negative results.

Recently 5 other moles have been found to be infected, *i.e.* 6 altogether out of 20 examined (30 %). The parasites in every case were present in such small numbers that a satisfactory stained preparation has not yet been obtained. A white rat was inoculated intraperitoneally with the heart-blood of one of the infected animals, but no infection followed.

Field-Vole (Microtus [Arvicola] agrestis).

12 of these have been examined with negative results.

II. Birds.

Trypanosomes of Birds.

It may be recalled that trypanosomes in birds have been found by Danilewsky (1885–1889) in Russia, by Dutton and Todd (1903) in the Gambia, by Hanna and Donovan (1903) in India, and by Ed. and Ét. Sergent (1904) in Algeria. Laveran and Mesnil (1904, p. 353) describe them as occurring in the owl (*Syrnium aluco*) in France, but it is interesting to note that they record their failure to observe them in a great number of other birds in that country. Levaditi, however, has discovered a trypanosome in the blood of *Padda orizyvora* obtained in Paris. An account of the structure of this organism is given by Laveran and Mesnil, and inoculation experiments have been carried out quite recently by Thiroux (1904) in the Pasteur Institute. Schaudinn's (1904) conclusions on the relationship between trypanosomes and endoglobular parasites were based on observations of the blood parasites of *Athene noctua* in Rovigno.

The examination of the blood of birds in this district proved negative with regard to the occurrence of trypanosomes; on the other hand, these parasites were found in several cases in the bone-marrow. The birds were mostly obtained in the months of June, July and September, and preparations of the blood and marrow were made within an hour or two of death. The following Table gives the results in a succinct form.

Thus out of 67 birds examined 11 had trypanosomes in the bone-marrow (16·4 %). In those cases in which they were present a careful search was necessary in order to detect them, and, indeed, in stained preparations it was almost impossible to find a specimen. The blood was in every case examined for trypanosomes and intra-corpuscular parasites but on no occasion were these observed.

The trypanosomes found in the marrow showed active movements when examined in normal salt solution; movements from place to place did not occur to any extent. In a fresh specimen from a blackbird several refractive granules were seen in the middle of the body of the parasite. A trypanosome found in a swallow appeared to be smaller than one observed at the same time from a blackbird.

The discovery of a *Spirochaete* in the blood of a martin and a trypanosome in its bone-marrow is interesting in the light of Schaudinn's researches. In support of his views regarding the relationship between

Bird	Number examined	Date	Result
House-Martin; *Chelidon urbica* (Linn.)	1	June	Spirochaetes in blood; a few trypanosomes in bone-marrow.
Song-Thrush: *Turdus musicus* (Linn.)	6	June, July, Sept., Nov., Dec.	3 examined in June and July had trypanosomes in marrow. 1 (28. 9) negative. 1 (22. 11) blood negative; one trypanosome seen in fresh preparation of marrow. 1 (19. 12) blood negative; one trypanosome seen in fresh preparation of marrow.
Blackbird; *Merula merula* (Linn.)	6	July, Sept., Nov., Dec.	1 (15. 9) blood negative; one trypanosome seen in preparation of marrow. 1 (25. 11. 4) blood negative; fair number of trypanosomes in marrow. Fresh preparations of liver, spleen, ovary, kidney, all negative. 1 in July; blood and marrow negative. 1 (17. 11) ,, ,, ,, ,, 1 (26. 11) ,, ,, ,, ,, 1 (12. 12) ,, ,, ,, ,,
Swallow; *Hirundo rustica* (Linn.)	11	June, July, Sept.	1 (15. 9) a very few trypanosomes in marrow. 5 in June—July; blood and marrow negative. 3 (14. 9) ,, ,, ,, ,, 2 (22. 9) ,, ,, ,, ,,
Chaffinch; *Fringilla coelebs*	1	26. 11. 4	Blood negative; a few trypanosomes in marrow.
Yellowhammer; *Emberiza citrinella*	1	16. 11. 4	,, ,, ,, ,, ,, ,,
House-Sparrow; *Passer domesticus* (Linn.)	27	June, July, Sept., Nov.	4 in June—July; blood and marrow negative. 2 (15. 9) ,, ,, ,, ,, 16 (21. 9) ,, ,, ,, ,, 1 (9. 11) ,, ,, ,, ,, 4 (16. 11) ,, ,, ,, ,,
Starling; *Sturnus vulgaris* (Linn.)	8	June, July, Sept., Nov., Dec.	5 in June, July, Sept.; blood and marrow negative. 1 (28. 11) ,, ,, ,, ,, 1 (12. 12) ,, ,, ,, ,, 1 (19. 12) ,, ,, ,, ,,
Crow; *Corvus frugilegus* (Linn.)	4	June, July, Sept.	3, blood and bone-marrow negative. 1, ,, ,, ,, ,, ,,
Jackdaw; *Coloeus monedula* (Linn.)	2	June, July	,, ,, ,, ,, ,,

these two organisms he mentions a similar instance, viz., the *Spirochaete* and *Trypanosome* found by Theiler (1903) in ordinary red-water and Rhodesian red-water fever (African Coast Fever). The spirochaete was present in considerable numbers in the martin's blood when examined immediately after death. In the fresh specimen the organism appeared to have several spiral twists (4 or 5), but in the stained preparation not more than two. In a film of the blood stained by Leishman's method the protoplasm has a pale-blue tint with no chromatin staining. The average length is $6{\cdot}7\,\mu$, breadth $1{\cdot}5\,\mu$. The spirochaetes were not present in the bone-marrow and there were no intra-corpuscular parasites.

III. Fishes.

Trypanosoma danilewskyi.

19 goldfish were examined, chiefly in the early months of the year. Trypanosomes were found in all, though in such small numbers that prolonged examination of the fresh specimen was usually necessary.

In a hanging-drop preparation the movements were exceedingly active and very contorted. The protoplasm had a granular appearance. With Leishman's stain the protoplasm of the body has a pale-blue colour, and contains small unstained spaces and chromatin granules. The macronucleus is situated near the middle of the body and stains more feebly than the micronucleus. As regards measurements the length of one was $38{\cdot}4\,\mu$, of another $32\,\mu$, and of a third $48\,\mu$: the breadth varied from 2 to $3\,\mu$.

The blood of an infected fish was removed aseptically from the heart and pipetted into the condensation water of rabbit-blood agar. The tubes were then kept at room temperature. In 10 days a considerable number of trypanosomes were seen in a hanging-drop preparation. In shape they were curiously tadpole-like, contrasting with the slender form of the fresh specimen. Their movements were much less active than those in a fresh blood preparation. On the 11th day a group of 4 members was seen, and on the 12th day one with 5 individuals. On the 13th day the trypanosomes were much fewer. Sub-cultures were made but did not grow, though a considerable amount of inoculating material was added to the culture tubes. The shape of the trypanosomes, the feeble motility of their flagella, and the fact that they did not give rise to a sub-culture point to their being degeneration

forms, similar to those observed in cultures of *Trypanosoma lewisi* and *Trypanosoma brucei.*

Small leeches were occasionally found attached to the scales of the fish during the summer months, and as they were the only external parasite seen it is not unlikely (as has already been suggested) that the infection is conveyed by them.

The principal point of interest elicited by these observations lies in the fact that in a limited area so many animals of widely varying species should harbour trypanosomes. The following Table shows this clearly:

I. Mammals.

1. Bats:—(*a*) *Pipistrellus pipistrellus*, 8 examined, 3 positive.
   (*b*) *Pterygistes noctula*, 1 examined, negative.
2. Rats:—proportion of infected animals estimated at 30 %.
3. Wild Rabbits:—40 examined, 4 positive—(10 %).
4. Field-Vole (*Microtus agrestis*):—12 examined, all negative.
5. Mole (*Talpa europaea*):—20 examined, 6 positive—(30 %).

II. Birds.

67 examined, 11 positive—(16·4 %).
Trypanosomes were found in 6 out of 10 species.

III. Fishes.

19 Goldfish examined, all positive.

EXPLANATION OF PLATE VIII.

Fig. 1. *a—g*: Trypanosome of the bat—showing frequent contortion of the body of the parasite.

Fig. 2. Multiplication forms of *Trypanosoma lewisi* occurring in blood of naturally infected wild rat; the flagellum of the parent-cell is easily distinguishable.

Fig. 3. Spirochaete observed in blood of the house-martin; trypanosomes were present in the bone-marrow.

Fig. 4. Trypanosome of the rabbit—showing a close resemblance to *Trypanosoma lewisi.*

Fig. 5. Trypanosome found in blood of goldfish (*Tryp. danilewskyi*).

Fig. 6. Sketch of fresh preparation of trypanosome found in the bone-marrow of the blackbird.

In conclusion, the more noteworthy facts recorded above may be summarised as follows:

1. The trypanosome of the bat seems to be sharply defined in

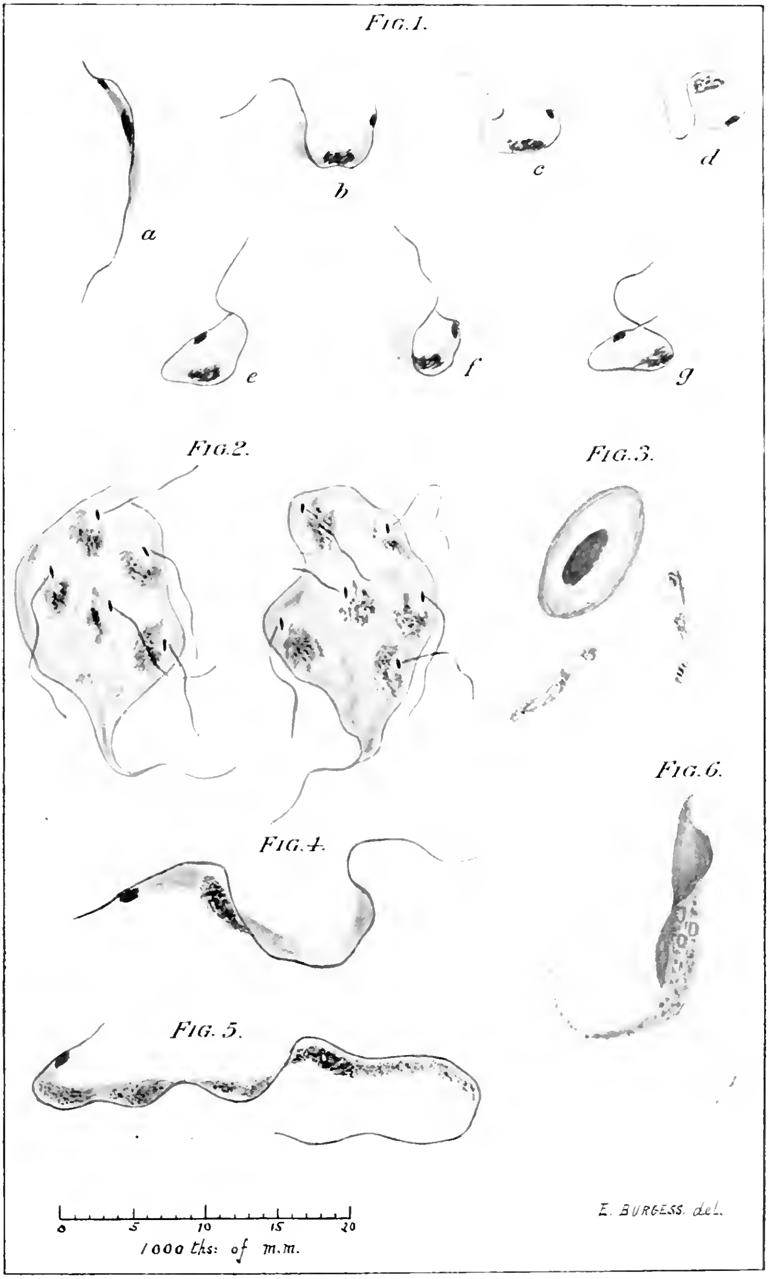
FIG. 1.
a
b
c
d
e
f
g
FIG. 2.
FIG. 3.
FIG. 4.
FIG. 5.
FIG. 6.
0 5 10 15 20
1000 ths: of m.m.
E. BURGESS. del.

several respects from trypanosomes hitherto described; chiefly from its small size and from its characteristic looped appearance in the stained specimen.

2. The wild rabbit, as in the case of the rat, is more frequently infected than the tame variety. Cultivation of the trypanosome for one generation at least presents no difficulty.

3. Blood-films from 2 wild rats with a natural infection of *Trypanosoma lewisi* in the stage of multiplication have been examined. The forms observed support the contention of Wasielewski and Senn and MacNeal that the mode of multiplication can be traced in every instance to an unequal longitudinal division.

4. A new species of trypanosome has been found in the blood of the mole.

5. Trypanosomes have been observed not infrequently in this locality in the bone-marrow of certain common birds but not in their blood.

6. No intra-corpuscular parasites have been observed in any of the birds examined.

7. In the case of one bird (*Chelidon urbica*, Linn.) a spirochaete in the blood was associated with a trypanosome in the bone-marrow.

I wish to express my thanks to Mr Oldfield Thomas, F.R.S., of the British Museum, who kindly identified the species of bats for me.

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A HANDY METHOD OF DETERMINING THE AMOUNT OF CARBONIC ACID IN AIR.

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Many years ago while making in the laboratory of the late Prof. Carnelley in Dundee, by Pettenkofer's method, a series of Carbonic Acid determinations, in which turmeric paper was used as the indicator, I observed that the last few brown rings made on the paper as the solution approached neutralization showed a tendency to become decolorized in a relatively short space of time, the final ones being seen to vanish even as one looked at them. This at the time—and subsequent observation has confirmed the truth of the conclusion—was ascribed to the CO_2 of the air rapidly neutralizing the solution of baryta, which getting more and more dilute as the solution of oxalic acid was gradually added, finally reached a degree of dilution that was all but instantly neutralized by the amount of CO_2 present in an ordinary atmosphere. Even then I came to the conclusion that the phenomenon depended on a principle which might probably be made the basis of a rapid method of estimating the amount of CO_2 in air generally, but it is only recently that I have had time to adjust the details of the method which I now propose. It will readily be understood that a long series of experiments other than those of which the results are now recorded were carried out before finality was reached on many points of the method. These need not be detailed here, though some of them will be alluded to as occasion arises. It may be stated that the method has been found to give results which approximate to the truth—at least as tested by Pettenkofer's method—much more closely than was at first anticipated.

The special recommendations of the method are, easiness and rapidity of execution, simplicity and inexpensiveness of apparatus, with equal simplicity of the calculations necessary to obtain a definite result.

Principle of the Method.

The method depends on the theoretical view that equal quantities of alkali in solution, when they present equal surfaces for absorption and are of equal depth, will be neutralized by the CO_2 in a given atmosphere in equal times: and by implication that surface, depth and strength of solution being constant while other conditions vary, the times necessary for neutralization will be inversely as the amounts of CO_2 present in the atmosphere to which they are exposed. Experiment shows that this is a very close approximation to the truth.

To put the principle in concrete form, it may be said that drops of alkaline solutions which are equal both as regards their diameters and the quantity of solution which they contain, whether of lime water, baryta, or magnesium hydrate, within the limits of strength indicated, if coloured by phenolphthalein, have their colour discharged in times which are practically in the inverse ratio of the amounts of CO_2 present in the several atmospheres to which they are exposed—that is to say, if one atmosphere contains twice as much as another it will decolorize equal-sized spots of these solutions, coloured as indicated, practically in half the time; if it contains three times as much it will decolorize them practically in one-third the time, and so on. If the diameters of the spots are unequal while the quantity of solution in the spots remains constant, the times of decolorization for the same atmosphere are inversely as the square of the diameters of the several spots, that is to say, inversely as their surfaces. This has been found from experimental data to hold good at least within the limits of experimental error.

The method therefore is simply this:—A number of as nearly as possible equal spots of one or other of these solutions, coloured as indicated, is exposed on a white surface to the atmosphere in which it is proposed to estimate the CO_2, and the time required for complete discharge of their colour noted. The CO_2 present in vols. in 10,000 parts is a function of the strength of the solution used, divided by the time in minutes taken for the discharge of the colour.

Stated generally the formula is:—

$$\frac{s \times f}{x} = \text{vols. of } CO_2 \text{ in 10,000 parts,}$$

where s is the strength of the solution stated in particular terms,

f is a factor which within the range of experimental error is fairly constant,

x is the time of colour discharge in minutes.

Solutions used, and their strength.

A solution of lime water, of baryta, or magnesium hydrate—for all these have been used in turn—coloured by the addition of a drop or two of solution of phenolphthalein, and of such strength that 100 c.c. of it has its colour discharged by ·5 to 2·5 c.c. of a solution of oxalic acid, every c.c. of which is equal to 1 mgrm. of CO_2, is used for the determinations. This latter solution is made by dissolving 2·8636 grms. of pure crystallized oxalic acid in a litre of distilled water. The strength of the lime water or other alkaline solution may, so far as experiment goes, lie anywhere between the limits indicated, but its equivalent in mgrms. and tenths of a mgrm. of CO_2 per 100 c.c. of solution must be exactly known—at least where it is intended to find the proportion of CO_2 in absolute vols. per 10,000 parts of air. If it is only intended to make a comparative estimate, as for example the amount in an internal atmosphere, in terms of the amount in outside air, it is not necessary to know the strength of the solution exactly, but it is still desirable that it should lie within the limits indicated. The strength of the solution should also be to some extent proportioned to the quantity of CO_2 to be estimated. Thus the CO_2 in outside air is best determined by a relatively weak solution, and a solution of magnesium hydrate of strength equal to ·5 mgrms. of CO_2 has been successfully used for this purpose. Stronger solutions take too long time for the discharge of colour and hence afford greater scope for the operation of other disturbing factors. On the other hand a solution of the strength indicated would have its colour discharged too quickly to admit of the correct determination of the time, by the most highly charged atmospheres. For example, spots of a solution equal to ·5 mgrms. of CO_2 per 100 c.c. of solution, of the average diameter, are decolorized by outside air in about 11 minutes, whereas an atmosphere containing 15 vols. in 10,000 parts would discharge the same spots in something less than $2\frac{1}{2}$ minutes. A little reflection will show that it is not expedient to reduce the time of exposure below about 10 minutes in any case, because any error in noting the time of discharge becomes relatively larger as the divisor becomes smaller, and the difficulty of accurate observation of the time is proportionately increased as the period of exposure gets shorter. In practice, at least where the same observer both puts out the spots and notes their time of colour discharge, it has been found impossible to estimate the time with greater accuracy than within half a minute. In the case of the more highly charged atmospheres it might be

possible sometimes to come closer, but for most part within half a minute is probably the nearest approximation that could be made by the most careful observer—the time, from the conditions of experiment, really not being capable of more exact determination. But even when the nearest full number of minutes is noted, provided that the number does not fall too low, say below 10, the results will be found sufficiently accurate for most purposes.

Quantity of solution in a 'drop' or 'spot,' and variations in the diameter of the 'spots.'

As regards the size of the drops it may be stated that the two 50 c.c. stoppered burettes with which the determinations were made both gave us nearly as possible 22 drops per c.c. (one drop = $\cdot 04\dot{5}$ c.c.) when discharging at the ordinary rate at which the spots are made. This is therefore considered the standard drop. There is no doubt considerable variation in burettes in this respect, but from an investigation which has been made on my behalf in regard to this point it would appear that there is likely to be no difficulty in procuring burettes of similar calibre to those which I used. It may be remarked that small deviations from the quantity indicated may be safely discounted, as drops of larger size on falling the same distance as smaller drops spread out to spots of larger diameter than the smaller, thus tending towards compensatory adjustment as regards their depth. As will be readily understood it is really the depth of the spot which is the important dimension from our present point of view. A drop of the standard size if it falls about $\frac{1}{2}$ inch gives a spot about 9 mm. in diameter. The tabulated results which accompany this paper, unless where otherwise stated, were made from the observation of spots of that diameter. Where the spots vary in diameter their times of discharge as before indicated are inversely as the square of their diameters, so that it is comparatively easy to reduce the time results obtained from the observation of spots of abnormal size to the time results of normal-sized spots. If we have spots say of 8, 9, and 10 mm. diameter respectively, but containing the same quantity of liquid, their times of discharge for the same atmosphere will be as the reciprocals of the squares of these numbers, or as $\frac{1}{64}$, $\frac{1}{81}$, $\frac{1}{100}$.

The following rule may be given for the reduction of the time of spots of abnormal diameter to the time of spots of normal diameter. If the spot is 8 mm. in diameter multiply the time by $\frac{64}{81}$; if 10 mm.

diameter multiply by $\frac{100}{81}$ to reduce their time to that of spots of 9 mm. diameter.

In practice, however, it has been found possible in the majority of instances to get over any difficulty arising from differences in the size of the spots, by carefully selecting spots of the particular size required and discounting all those of abnormal size. It has frequently been observed—more particularly as it appears in the case of outside determinations—that owing probably to variations of atmospheric moisture the surface tension of the drops varied from day to day. They would sometimes stand up like beads for several consecutive days together, at other times they flattened out to a greater or less degree. Still even here it was nearly always possible, either by gently tapping the edge of the plate on which they were exposed, or by careful selection, to get spots of the required diameter. It was further found that the plate had always to be free from adherent moisture, otherwise the spots ran, and were consequently of no use for the purpose of estimation. The slightest trace of grease on the surface of the plate is equally fatal to a successful result.

How to prepare and keep the working solution.

A solution may be prepared by diluting a saturated[1] solution of lime water about 50 times, say 20 c.c. up to a litre, with distilled water that has been boiled for some time and cooled to the exclusion of air, and then adding phenolphthalein solution till the maximum degree of coloration is attained. This solution is then standardized against a solution of oxalic acid, every c.c. of which corresponds to one mgr. of carbonic acid. To do this 100 c.c. of the coloured solution is placed in a porcelain basin and the oxalic acid run in drop by drop with constant stirring till complete decolorization is obtained. The process should be repeated till coincident results are obtained or the average of several determinations may be taken as the working strength of the solution. A very convenient solution may also be prepared by burning so much magnesium ribbon and allowing the resulting magnesia to drop into a flask of water which has been boiled for some time and allowed to cool with exclusion of air. This solution coloured

[1] Lime water of the shops is very variable in strength. I found it to vary so that 25 c.c. of solution ranged from 16·5 to 26·1 c.c. of oxalic acid, 1 c.c. of which was equal to one milligramme CO_2. Normal strength is about 20 c.c. for distilled water and pure lime at ordinary temperatures.

with phenolphthalein forms a convenient working solution and after standing for a few days with occasional shaking is found to be just about the required strength. It must of course be kept closely stoppered. It was at first hoped in this way to obtain a solution of constant strength, but results show that this cannot be depended on with certainty. It has therefore to be always standardized against the oxalic acid solution. Solutions of magnesium hydrate in strength from ·5 to 2·5 mgr. of carbonic acid per 100 c.c. have been made and used in this investigation.

The only apparatus required is a stoppered burette and a white glazed stoneware plate.

The burette is washed out with some of the solution and is then filled from the remainder. To the open end of the burette is fitted a small U-tube containing soda-lime to prevent the entrance of carbonic acid. For additional protection the distal end of this tube when the apparatus is not in use may be closed with a tight-fitting rubber stopper. Thus protected the solution is maintained of the same strength for a considerable time, and a single burette full of solution is good for a large number of determinations.

Technique of method.

To make a determination, the rubber stopper is removed from the distal end of the protecting U-tube. The few drops of solution in the beak of the burette are then run out, because the carbonic acid of the air having had access to this portion of the solution has reduced its strength. Then, holding the beak of the burette about $\frac{1}{2}$ inch above the plate, its long axis as nearly vertical as possible, the operator gradually turns the stopper and allows the solution to fall, but not too quickly, in drops from the burette while he at the same time gradually moves it over the surface of the plate. Ten or a dozen spots will usually suffice. They take less than as many seconds to put out. The number of minutes from the time the last spot is put out onwards to complete decolorization of all the spots has now to be noted. The slight difference in their exposure periods, arising from the fact of the spots being not simultaneously but successively made, is, as will be inferred from what has already been said, of no consequence and is accordingly discounted. Immediately after recording the time of commencement of the period of exposure of the spots, the observer proceeds to note whether any material difference in the diameter of the spots exists; and if there be any decided difference he may as alternative measures, either (1) expunge the extremes,

that is to say those that vary most from the average 9 millimetre diameter—this facilitates observation of the normally-sized spot; or (2) he notes separately the time for 8 millimetre spots, for 9 and 10 millimetre spots respectively, and calculates out the result separately for each set according to the process of reduction already stated. Observation of the normally-sized spots and of either the 8 or the 10 millimetre spots, not both, will usually be found as much as one can conveniently attend to. Where two results have been calculated out from the discharge periods of different-sized spots, they have generally been found to show a remarkable coincidence. The process by expunging the abnormal-sized spots is, however, the one to be recommended in the first instance. (3) At first from 40 to 60 spots were put out and the time of discharge, as they became decolorized in batches, noted. The average time per spot was then calculated out and taken as the mean time of discharge. This method was mostly used for outside air, the time for inside atmospheres of any intensity as regards their charge of CO_2 being often too short, and the time for the various spot groups often falling too closely together, to admit of accurate observation.

Examples will now be given of the different methods of recording the period of discharge of the spots.

(1) Spots 9 millimetres in diameter of a solution of lime water equal to 1·5 milligrammes of carbonic acid per 100 c.c. of solution, required a uniform period of 17 minutes to discharge their colour in a certain atmosphere. This according to the scheme of calculation which was afterwards worked out gave 6·08 vols. of carbonic acid per 10,000. A determination by Pettenkofer's method, made as nearly as possible simultaneously, gave 6·3 vols.

(2) Spots of 9 millimetres diameter of a solution of magnesium hydrate which was equal to 2·5 milligrammes of carbonic acid per 100 c.c. required 17 minutes, while spots of 8 millimetres diameter required 21 minutes.

The 9 millimetre spots took 17 minutes, and gave by calculation 10·12 vols. of CO_2.
" 8 " " " 21 " " " " " 10·37 " "

Pettenkofer's method gave 10·06 vols.

(3) In determining the carbonic acid in outside air on one occasion 50 spots of a solution of lime water equal to 2·1 milligrammes of CO_2 per 100 c.c. were put out and gave the following results as regards time of discharge:—

4	spots required	34	minutes	$34\times 4=$	136,
11	" "	38	"	$38\times 11=$	418,
18	" "	40	"	$40\times 18=$	720,
5	" "	45	"	$45\times 5=$	225,
12	" "	53	"	$53\times 12=$	636,
				50	2135,

$\frac{2135}{50}=42{\cdot}7$ minutes as average time of discharge.

This according to the method of reduction would be equivalent to 3·67 vols. of CO_2 in 10,000.

Determination of Factor for Reduction of Results.

For the purpose of determining what relation obtained between the strength of solution and the time of exposure on the one side and the value of CO_2 in parts per 10,000 on the other, a large number of determinations both in outside air (21) and in indoor atmospheres (77) were, as far as the essential differences in the methods allowed, simultaneously made by this method and by Pettenkofer's method, and the results as obtained by the latter method were taken as the basis for determining the relation subsisting between these three functions according to the formula:

$$\frac{\text{Strength of Solution (in milligrammes of } CO_2 \text{ per 100 c.c.)}^{(1)} \times \text{Constant}^{(2)}}{\text{Time of Colour discharge in minutes}^{(3)}} = \text{vols. of } CO_2{}^{(4)} \text{ in 10,000 parts.}$$

That is to say, in a given case (1), (3), and (4) being known, it was required to find (2), the constant factor.

Example. A solution of lime water equal to 1·5 mm. of CO_2 in 10,000 requires 17 minutes to decolorize in an atmosphere which was shown by Pettenkofer's method to contain 6·3 vols. of CO_2 in 10,000. Find the factor or constant (2) in this case:

Here $$\frac{1{\cdot}5 \times \text{factor}^{(2)}}{17} = 6{\cdot}3.$$

Whence $$\text{factor}^{(2)} = \frac{6{\cdot}3 \times 17}{1{\cdot}5} = 71{\cdot}4.$$

In this way the factor was obtained in every case throughout the 21 outside cases and also throughout the 77 cases of inside atmosphere, and the corresponding factors are tabulated in the accompanying tables.

The average factor in the case of

21 determinations in outside air was found to be 74·6 for unreduced Pettenkofer results and 80·5 when reduced to normal temperature and pressure;

57 determinations in ordinary inside atmospheres was found to be 68·8 for unreduced and 73·9 for reduced results;

20 determinations in highly vitiated inside atmospheres was found to be 70·5 for unreduced and 78·8 for reduced.

The probable explanation of these differences will be considered later. The first factor (74·6) was provisionally used for the reductions of outside air generally, and the second (68·8) for ordinary inside atmospheres.

When the results in the case of inside atmospheres are thrown into groups according to the amount of carbonic acid present, some little difference in the factors was shown to obtain, probably pointing to some divergence from the strict proportional ratio from atmospheres of less to atmospheres of greater intensity as regards the amount of CO_2 present.

Again, solutions of lime water, baryta, and magnesium hydrate of various strengths, within the limits indicated, were used for the determinations, and on calculating out the average factor for each substance no grounds have been obtained for preferring the solution of one substance to that of another, as very little difference in the average factors was disclosed. What difference does obtain in the case of one of the solutions may be ascribed to some little deviation of the strength of the solution from that actually recorded against it.

Thus lime water used in 4 different strengths gave as the average of 30 determinations in ordinary inside air 68·81;

baryta used in one strength gave as the average of 10 determinations 70·59;

magnesium hydrate used in 4 different strengths gave as the average of 17 determinations 68·3.

It may be concluded therefore that 68·8 is a sufficiently approximate factor for the reduction of ordinary inside atmospheres and 74·6 for outside air. It should be stated, however, that some difference in these figures may be expected to arise as the result of personal equation, but this is not likely to be great.

Before going on to cite a few examples it may be well to give a general formula to facilitate the calculation of results. This formula is

$$\frac{s \times c}{x} = CO_2 \text{ in vols. in } 10{,}000 \text{ parts,}$$

where $s =$ the strength of solution in mgrs. of carbonic acid per 100 c.c. of solution,

$c =$ the constant—74·6 for outside air; 68·8 for ordinary inside atmospheres.

$x =$ the number of minutes required for discharge of colour.

Examples—first, when solutions of different alkalis have been used.

(1) Spots of normal size of a solution of lime water equal to 1·5 milligrammes of carbonic acid per 100 c.c., in a certain atmosphere took 9 minutes to decolorize. What was the amount of CO_2 in vols. per 10,000 present?

Calculation $$\frac{1{\cdot}5 \times 68{\cdot}8}{9} = 11{\cdot}47 \text{ vols. } CO_2 \text{ in } 10{,}000.$$

By Pettenkofer's method the same atmosphere gave 11·56 vols.

(2) Spots of normal size of a solution of magnesium hydrate equal to ·5 milligrammes of CO_2 per 100 c.c. required $3\frac{1}{2}$ minutes to decolorize in a certain atmosphere. What was the amount of CO_2 per 10,000 present?

Calculation $$\frac{{\cdot}5 \times 68{\cdot}8}{3{\cdot}5} = 9{\cdot}83 \text{ vols.}$$

By Pettenkofer's method this was found to be 9·66 vols.

(3) Normal-sized spots of a solution of baryta equal to 1·7 milligrammes of CO_2 in 100 c.c. of solution required 18 minutes for complete decolorization. What was the content of CO_2 in parts per 10,000 vols. of the atmosphere under examination?

Calculation $$\frac{1{\cdot}7 \times 68{\cdot}8}{18} = 6{\cdot}5 \text{ vols. } CO_2.$$

Pettenkofer's method gave 6·52 vols.

The following two examples will suffice for spots of abnormal size:—

(1) Spots of 8 millimetres diameter of a solution of magnesium hydrate equal to 1·8 milligrammes of CO_2 in 100 c.c. required 34 minutes to decolorize. What was the content of CO_2 in parts per 10,000 in the atmosphere under examination?

Calculation $$\frac{1{\cdot}8 \times 68{\cdot}8}{\frac{64}{81} \times 34} = 4{\cdot}61 \text{ vols.}$$

By Pettenkofer's method this was equal to 4·43 vols.

(2) Spots of 10 millimetres diameter of a solution of magnesium hydrate equal to 2·5 milligrammes of CO_2 per 100 c.c. required 17 minutes to decolorize. What was the content of the atmosphere as regards CO_2 in parts per 10,000?

Calculation $$\frac{2{\cdot}5 \times 68{\cdot}8}{\frac{100}{81} \times 17} = 8{\cdot}195 \text{ vols.}$$

By Pettenkofer's method this was equal to 8·14 vols.

In the appended tables the rough rule of simply increasing by $\frac{1}{4}$th the observed time for 10 mm. spots and of decreasing by $\frac{1}{5}$th the observed time for 8 mm. spots to obtain the time for 9 mm. spots was the one followed. Hence some little discrepancy will be found between the above results and those there given. The procedure now indicated is probably sufficiently accurate for practical purposes and is certainly much simpler.

So much for inside atmospheres; an example will now be given in the case of outside air:—

Example. Spots of a solution of magnesium hydrate equal to ·5 milligrammes of CO_2 in 100 c.c. required 12 minutes to decolorize. What was the volume of CO_2 in the air?

Calculation $$\frac{\cdot 5 \times 74 \cdot 6}{12} = 3 \cdot 11 \text{ vols.}$$

By Pettenkofer's method this was found to be 3·08 vols.

The difference of factor employed for outside air should be noted.

Where the observer is not in a position to determine the strength of his working solution by standardizing it against oxalic acid of known strength, he may still make comparative determinations of the amount of carbonic acid in one atmosphere in terms of that in another, *e.g.* of one inside atmosphere in terms of that in another inside atmosphere, or of an inside atmosphere in terms of that in external air; but in comparing outside air with an inside atmosphere the difference in the factors for reducing these two groups should not be forgotten, and the average amount of carbonic acid in outside air should be taken with some approach to accuracy. It appears to be necessary to insist that this is not always, or even frequently, 4 parts, but usually about 3·4 parts in 10,000, as determined by Pettenkofer's method. Of 23 determinations made in Elgin the average was 3·45 vols.

Example. Spots of a solution of indefinite strength gave for outside air 39 minutes; for an internal atmosphere 14 minutes for colour discharge. What was the probable amount of carbonic acid in 10,000 parts in the latter atmosphere?

Calculation $$\frac{39 \times 68 \cdot 8 \times 3 \cdot 45}{14 \times 74 \cdot 6} = 8 \cdot 86 \text{ vols.}$$

Pettenkofer's method gave 9·35 vols.

Again, the content of outside air as regards CO_2 being known both in terms of this method and of Pettenkofer's method, the content of any other atmosphere of which the discharge period for the same solution is known, can be calculated from the data indicated.

Example. Outside air containing 3·08 vols. in 10,000 parts according to Pettenkofer's method required 12 minutes to decolorize normal-sized spots. What would be the volume of CO_2 in an atmosphere which decolorized the spots in $3\frac{1}{2}$ minutes?

Calculation $$\frac{12 \times 68 \cdot 8 \times 3 \cdot 08}{3 \cdot 5 \times 74 \cdot 6} = 9 \cdot 74 \text{ vols.}$$

Pettenkofer's method gave 9·66 vols.

It hardly needs to be stated that the results by this method of

procedure are not calculated to attain the same degree of accuracy as those that are directly deduced from the ascertained strength of the solution and the exposure period for the given atmosphere.

One application to which the present method lends itself with marked facility may be mentioned, and that is for indicating when the limits of healthy ventilation are being exceeded. For instance it may be asked how by this method could it be shown when the limit of 9 vols. per 10,000 was being reached or exceeded in a given case? This in practice simply amounts to determining the number of minutes that will be required by an atmosphere containing 9 vols. of CO_2 to discharge spots of a solution of given strength. This may be found from the formula:

$$\frac{s \times 68{\cdot}8}{x} = 9.$$

If the strength of the solution be taken at, say, 2 mgrms. of CO_2 per 100 c.c. then

$$\frac{2 \times 68{\cdot}8}{x} = 9, \text{ whence } x = 15{\cdot}3 \text{ minutes.}$$

If then, spots of normal size, of a solution of the strength indicated, take 15 or any less number of minutes to discharge, the limit of 9 vols. in 10,000 is being exceeded. Similarly, the time for any other limit, the strength of solution being known, may be determined. But if the strength of the solution is not known, then the time taken by outside air becomes the standard; and of this an average of a considerable number of determinations should be taken and used as the working value. Say that it takes y minutes for 3·4 vols. per 10,000 in external air, what will be the relation between x and y when the result is 9 vols. of CO_2 in 10,000?

Calculation $$\frac{y \times 68{\cdot}8 \times 3{\cdot}4}{x \times 74{\cdot}6} = 9.$$

Whence $$x = \frac{y}{2{\cdot}8} \text{ nearly.}$$

That is to say, the limit of 9 vols. is being exceeded when the time taken by outside air is more than 2·8 times as great as the time taken by the atmosphere under examination.

Physics of the method.

It need hardly be said that it was naturally anticipated that in consequence of the freer movements of the air, outside determinations

would show a much quicker rate of discharge than those in internal atmospheres. That this was not the case is, and remains, the paradox of the investigation. There cannot be a doubt however that as regards the discharge of colour the conditions in the two sets of atmospheres are quite dissimilar. Inside, where the movements of the air are necessarily at a minimum, it will be seen, if the spots are closely watched, that as the period of exposure advances, the colour moves in from the edge—and presumably also from the surface—of the spot, so as eventually to show a colourless margin all round it. This colourless margin broadens as time goes on, till finally, just before complete neutralization, a minute point of colour, often, to the last, of the same general intensity of colour as the original spot, is left at its centre. The final vanishing of this point of colour in the case of internal atmospheres gives a very definite and precise end-reaction. In the case of outside air, on the other hand, except on all but the calmest days, this marginal process of colour discharge is not observed. In consequence of the relatively greater movement of the air, the general mixing of the different layers of the spots causes the colour to be maintained of a uniform though gradually fading tint throughout the spot up to the moment of complete colour discharge. The end-reaction for this reason is in this case very much less definite and precise. But it must not be inferred that this fact has in any way led to a general overstating of the time of discharge. Rather the reverse was the case: for I found there was a general tendency on my part to consider the reaction as complete when just the faintest perceptible trace of colour remained. For the reasons just given I am strongly inclined to believe that the difference in the rate of discharge in the two cases is intimately related to the different physical conditions that obtain. These I now proceed to discuss.

In the case of internal atmospheres, three elemental processes appear to be involved in bringing about the discharge of colour. These are (1) Solution of the carbonic acid in the surface layers of the spot, (2) Neutralization of the alkali by the carbonic acid so dissolved, (3) Diffusion of the carbonic acid subsequently dissolved into the deeper layers of the spot, with further neutralization of the alkali in these layers. Conceiving the spot, as made up of a series of concentric shells we may picture the process of colour discharge somewhat as follows: A quantity of carbonic acid proportional to what exists in the atmosphere immediately in contact with the spot is dissolved in its surface layer, and proceeds to neutralize the alkali in it, which done, the surface layer

dissolves a similar quantity of carbonic acid from the atmosphere, which thereafter passes on by diffusion into the second layer and there neutralizes the alkali. It is succeeded in the surface layer by a further proportional quantity of carbonic acid, which also passes on by diffusion to the deeper layers as they are successively neutralized, to be succeeded by another in the surface layer and so on. In this way we may conceive the spot gradually invaded by a series of waves of carbonic acid, starting from and taking up their intensity of change from the quantity of carbonic acid present in the atmosphere, the first wave always neutralizing the alkali, the next passing on into its place by diffusion, and proceeding to neutralize another layer of alkali, and so on till the whole is neutralized and the total colour discharged. If these several changes could be supposed to go on simultaneously, continuously, and *pari passu*—not successively as described—we should I think have a fairly complete picture of what takes place in a spot. Of these three processes the general time rate of discharge is no doubt determined by the rate of solution of the carbonic acid and the rate of neutralization of the alkali conjointly,—the one rate necessarily being a function of the other. The rate of diffusion very evidently depends on and is limited by the rate of neutralization, as the carbonic acid cannot possibly diffuse through a layer of alkali, that is to say, the alkali must first be neutralized before the carbonic acid can pass on. For this reason the diffusion in a spot must take place at a slower rate than it would in water or any simple neutral solution. But it is evident that it must, at least within the limits of experiment, have always been sufficient to keep a proportional quantity of carbonic acid in contact with the particular layer of the spot in which neutralization was taking place.

It will also be apparent on this view of the physics of the method of colour discharge that an excess of carbonic acid must exist in the surface layers of a spot at and before the moment of complete discharge. That this is the case may be demonstrated by shaking the plate on which the spots are exposed just as they are nearing their final stage, when the discharge of the remaining colour will be seen to be perceptibly accelerated by the coloured area still remaining being brought into contact with the carbonic acid dissolved in the colourless surface layers.

In the case of outside determinations on the other hand (1) solution and (2) neutralization are confined to a hypothetical surface layer, and the effect of this surface solution and neutralization is immediately distributed through the liquid of the spots by (3) convection currents,

arising from the movement of the liquid of the spot, such movement being impressed on it by the movements of the air with which it is in contact. In consequence of the mixing of the different layers of the spots, the repeated fractional bleachings that take place in the surface layer have their general effect distributed through the total liquid of the spot, thus leading finally by imperceptible degrees to total bleaching of the same. The liquid of the spot is, as it were, rotated by the movements indicated. Coming in contact with the air in the surface layer, it absorbs a quantity of carbonic acid proportional to the amount present in the air, its alkali is neutralized, when it is forthwith carried off to be succeeded by another portion of the liquid of the spot which is similarly affected. This again is succeeded by another and another portion of the liquid with the same result till finally the colour of the spot is entirely discharged. This process, it will be seen, is very different from that described as probably obtaining in the case of spots exposed to internal atmospheres. Whether this movement of the liquid in thus limiting the action of the carbonic acid to the surface layer of the spot has any effect in retarding the general rate of solution of the carbonic acid —and as a consequence the general rate of neutralization of the alkali also—may be left an open question, but it certainly strikes one as probable that the process of mixing of the liquid of the various layers of the spot must take some time for its accomplishment, and hence naturally lead to a general increase in the length of time required for colour discharge. In any case if the rationale of the various changes that take place in the spots is on the lines just indicated, it will be very evident that a different rate of discharge in the two cases is extremely probable.

I should also be inclined to think that movement of the liquid of the spot rather than movement of the air over it—if it were possible to dissociate the two in fact—is really the important element in the case. The movement of the air containing carbonic acid can hardly be conceived as sensibly affecting the solution of the carbonic acid in the liquid of the spot, provided that air containing the same constant quantity of carbonic acid is always in contact with it: whereas the constant movement of the liquid in which this solution takes place, or the continued replacement of one portion of the liquid which has been momentarily in contact with the air by another portion, may have an important bearing on the quantity of carbonic acid dissolved. Neutralization of the alkali, depending as it does entirely on the quantity of carbonic acid dissolved, would necessarily vary with the rate of solution.

Experiments are in progress which have for their object the finding of specific answers to some of the points just raised.

A uniform factor suggested.

It is well known as a matter of fact that, notwithstanding the possibility of numerous individual errors arising from a variety of causes—which need not be detailed here—Pettenkofer's method gives results which are on the whole too high. This may be illustrated by a reference to the results obtained for external air. The careful experiments of Reiset and others on the Continent, and those of J. S. and E. S. Haldane[1] in this country by the gravimetric method, give an average for outside air of just under 3 vols. in 10,000.

Pettenkofer's method gives an average of 3·5 vols. 3·45 was found by me as an average of the determinations in Elgin by Pettenkofer's method. Dr Angus Smith's average for the air of Scotland generally was 3·36 vols. It is very evident that Pettenkofer's method cannot be put in competition with any carefully executed determination by a gravimetric method. Hence it must be concluded that Pettenkofer's method gives results which are about ·5 vols. too high. For this reason the factors we have deduced for this method ought, doubtless, to be proportionally reduced. If the factor 74·6 for outside air is reduced in the ratio indicated, it will be found to give 63·9 as the corresponding factor. The average all over for ordinary internal atmospheres is 68·8. In specially vitiated atmospheres it was 70·5. These latter factors would also be subject to some reduction for the average error of Pettenkofer's method. As all these variations of the reducing factor practically fall within the limits of estimational error of the individual analyses, I think on the score of practical expediency the factor 68·8 should be used all over, and I am further of opinion that no great loss of precision is likely to result in consequence. Only in cases where special accuracy is desirable, as in reducing the averages of large numbers of determinations, ought special factors to be used.

Something may be said as to how far the results obtained by the present method and by Pettenkofer's method are capable of comparison. They are not strictly comparable. This method estimates the amount of carbonic acid over the whole period of exposure of the spots, and the results as determined by it may be taken as giving the average amount of carbonic acid during that period. Pettenkofer's method on the other

[1] *This Journal*, vol. II. p. 421, 1902.

hand gives the amount of carbonic acid at the moment of collection of the specimen. Generally speaking the sample for estimation by Pettenkofer's method was taken some time during the exposure of the spots. This of course would rarely, if ever, be coincident with the mean period of the spots, and especially when it is considered that the determinations were made in atmospheres which were for the most part undergoing rapid increase as regards their carbonic acid, some discrepancy between the results as obtained by the two methods might reasonably be expected. The discrepancy was certainly greater when the interval between the determinations by the two methods was greatest. Again, the determinations were not always carried out under similarity of conditions as regards place. The following example will show what is meant. A determination at 9.10 a.m. in the laboratory by the spots at the height of the working bench gave 3·65 vols. A sample taken at the same time at the height of the collecting jar above the floor gave by Pettenkofer's method 6·42 vols. Two hours later the spots gave 5·7 vols. at the height above the floor at which the sample for Pettenkofer's method was taken. Here there was distinctly indicated the existence of a stratum of air highly charged with carbonic acid extending along the floor, but which evidently did not reach to the level of the working bench and had not been dispelled—at least to any great extent—two hours later. Hence arose the discrepancy of the results at the two levels and by the two methods.

On the whole it may be said that the method now described gives more reliable results than could have been anticipated on theoretical grounds, and though it is not propounded as a method of scientific precision, I am disposed to think it will be found useful and sufficiently exact for most purposes. Its very evident simplicity as regards apparatus, manipulation, and the subsequent calculation of results, strongly recommend it. It is believed from the ease with which the necessary apparatus can be carried from place to place, and the results worked out on the spot, that it will be found of much service for the informal sampling of air as regards its content of carbonic acid, and of indicating where other methods, if thought necessary, may be brought in to clinch the result. In the hands of an intelligent factory manager or schoolmaster I believe it will be found capable of producing results of the greatest value from a hygienic point of view.

TABLE I. *Ordinary Internal Atmospheres.*

Average Factor for Reduction, 68·8.

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.
Date	Time	Place	Capacity of Room	Time taken by "Spots" x	CO_2 in vols. per 10,000 By "Spots" $\frac{S\times 68\cdot 8}{x}$	CO_2 in vols. per 10,000 By Pettenkofer P	Pettenkofer reduced to 760 mm. and 0° C.	Calculated factor $\frac{S\times f}{x}=P$	Strength of solution in milligrammes of CO_2 per 100 c.c. $=S$	Remarks
Sept. '04				Minutes						
16th	8 p.m.	Dining Room	3200 ft.	17	6·08	6·3	6·82	71·40	$CaH_2O_2=1\cdot 5$	4 persons present. 3 gas-jets for 1 hour.
	9 ,,	,,	,,	11	9·38	9·27	10·06	67·98	,,	
	9.56 ,,	,,	,,	9	11·47	11·56	12·57	69·36	,,	
18th	7.30 ,,	,,	,,	12	8·60	8·07	8·52	64·56	,,	
	8.30 ,,	,,	,,	10	10·30	10·79	11·45	71·93	,,	
19th	8.10 ,,	,,	,,	14	9·09	9·01	9·64	68·72	$CaH_2O_2=1\cdot 85$	
20th	8.14 ,,	,,	,,	14	9·09	9·35	10·11	70·76	,,	Ventilation in the interval.
	9.30 ,,	,,	,,	13 for 10 m.	7·83	6·97	7·57	60·29	,,	
21st	8.30 a.m.	Bedroom	2400	21½	5·92	5·32	5·63	61·83	,,	2 persons present for 9 hrs. Window open, door ajar.
	8.40 p.m.	Dining Room	3200	9	10·70	9·66	10·50	62·10	$CaH_2O_2=1\cdot 4$	Simultaneously determined by solutions of CaH_2O_2 and MgH_2O_2 of strengths indicated.
	,, ,,	,,	,,	3½	9·83	,,	,,	67·62	$MgH_2O_2=\cdot 5$	
	10.15 ,,	,,	,,	7½	12·84	12·58	13·59	67·40	$CaH_2O_2=1\cdot 4$	
22nd	8.40 a.m.	Bedroom	2400	18	5·35	5·69	6·02	73·16	,,	2 persons for 9 hrs. Window open, door ajar.
	7.30 p.m.	Dining Room	3200	11	8·75	9·06	9·65	71·19	,,	
	8.30 ,,	,,	,,	9	10·70	10·71	11·47	68·85	,,	
	10.15 ,,	,,	,,	9½	10·14	10·20	10·95	69·22	,,	
23rd	8.45 a.m.	Bedroom	2400	17	5·67	5·75	6·04	69·82	,,	2 persons for 9 hrs. Window open, door shut.
	7.21 p.m. S. 7.45 ,, P.	Dining Room	3200	14	6·88	7·67	8·19	76·70	,,	
	8.30 ,,	,,	,,	10	9·63	8·92	9·55	63·72	,,	
	9.12 ,,	Laboratory	870	12	8·03	8·24	8·75	70·63	,,	
24th	8.25 a.m.	Bedroom	2400	19	5·07	4·84	5·09	65·69	,,	2 persons for 9 hrs. Window open, door ajar. Strong wind blowing in at window.
	7.38 p.m.	Laboratory	870	15	6·42	6·29	6·60	67·40	,,	

25th	2.30 p.m.	Laboratory	870	15	8·26	7·82	8·36	65·17	$MgH_2O_2=1·8$	Distilling water all day. Gas lighted in the interval. Moist atmosphere. Temp. latterly 68° Fah.
	5.40 ,,	,,	,,	14	8·84	8·50	9·25	66·11	,,	
	6.45 ,,	,,	,,	9	13·76	14·62	16·02	73·10	,,	
	7.25 ,,	,,	,,	8	15·48	Not det.	—	—	,,	
26th	8.30 a.m.	Bedroom	2400	26	4·76	4·60	4·91	66·44	,,	2 persons present for 9 hrs. Door and window open.
	7.20 p.m.	Dining Room	3200	15	8·26	8·38	8·96	69·83	,,	
	8.30 ,,	,,	,,	14	8·84	8·50	9·13	66·11	,,	
	9 ,,	Laboratory	870	15½	7·99	7·82	8·36	67·33	,,	
27th	4.30 ,,	,,	,,	34 for 8 m.	4·66	4·43	4·72	72·50	,,	
			Spots on Bench		3·65				,,	
28th	9.10 a.m.	,,	Pk. Height of jar above floor			6·42	6·72			
	11.10 ,,	,,	Spots later	18	5·72				$CaH_2O_2=1·5$	
	3.20 p.m.	Consulting Room	1750	19½	5·29	5·11	5·44	66·43	,,	Unventilated for several hours.
	7.15 ,,	Dining Room	3200	8	12·90	13·09	13·95	69·81	,,	
	9.15 ,,	,,	,,	7	14·74	14·61	15·71	68·18	,,	
29th	8.15 ,,	,,	,,	10	10·32			64·60	,,	
	8.30 ,,	,,	,,			9·69	10·32	74·28	,,	Ventilation in interval.
	8.45 ,,	,,	,,	11½	8·97				,,	
30th	4 ,,	Consulting Room	1750	33	3·14	3·23	3·38	71·06	,,	Free ventilation by open window.
	10 ,,	Dining Room	3200	8	12·9	13·25	14·10	70·66	,,	
Oct. 1st	7 ,,	,,	,,	11½	8·97	8·58	9·10	65·78	,,	
	7.50 ,,	,,	,,	9	11·47	11·78	12·38	70·68	,,	
2nd	6.35 ,,	,,	,,	20	6·88	7·18	7·67	71·88	$MgH_2O_2=1·8$	
3rd	9 ,,	,,	,,	15	9·19	9·85	10·63	73·88	,,	
4th	9.20 ,,	,,	,,	18	7·64	6·97	7·50	62·73	,,	
5th	9.15 ,,	,,	,,	13½	8·66	8·10	8·65	64·80	$BaH_2O_2=1·7$	
6th	8.20 ,,	,,	,,	16	7·31	7·65	8·29	72·00	,,	
7th	2.45 ,,	,,	,,	35 for 8 m.	4·18	4·5	4·82	74·11	,,	4 persons present for an hour. No gas.
8th	,, ,,	,,	,,	15	7·80	8·34	9·08	73·59	,,	
10th	,, ,,	,,	,,	25	4·68	4·79	5·16	70·44	,,	4 persons present for an hour. No gas.
	7.40 ,,	,,	,,	12	9·75	10·06	10·83	71·01	,,	
	8.55 ,,	,,	,,	8½	13·76	14·28	15·36	71·40	,,	
11th	9.40 ,,	,,	,,	18	6·50	6·52	7·02	69·03	,,	
12th	7 ,,	,,	,,	15	7·80	8·15	8·79	71·91	,,	
	9.5 ,,	,,	,,	13 for 8 m.	11·24	11·05	11·98	67·60	,,	
	10.2 ,,	,,	,,	17	10·12	10·35	11·27	70·38	$MgH_2O_2=2·5$	
17th	7 ,,	,,	,,	20	8·6	8·14	8·86	65·12	,,	
				17 for 10 m.	8·1			69·19	,,	
19th	6.50 ,,	,,	,,	17	10·12	10·06	10·86	68·41	,,	
				21 for 8 m.	10·37			67·58	,,	

TABLE III. *Results in Specially Vitiated Atmospheres.*

Date	Place	Time of start	Strength of solution in milligrammes CO_2 per 100 c.c.	Time of Spots		Carbonic acid vols. in 10,000.					Remarks
						"Spot" factor 68·8			Pettenkofer		
				First (Normal 9 mm.)	Last (Normal 8 mm.)	First	Last	Average	Unreduced	Reduced to 760 mm. and 0° C.	
1905				Minutes	Minutes						
Jan. 12th	Consulting Room 1750 ft.	Shut up at 4.50 p.m. 6.10 p.m.	CaH_2O_2 4·1	8	9	35·26	39·67	37·47	37·80	42·92	Two gas brackets. Damper down. Gas stove.
		8 ,,	,,	7	10	40·29	35·70	37·99	37·06	42·02	
14th	Dining Room 3200 ft.	5.20 ,,	,,	23	29	12·26	12·31	12·29	13·86	14·98	18 children, 3 adults. 3 gas-jets for ½ hr. Pk. sample taken as spots started.
,,	Drawing Room 3200 ft.	8.30 ,,	,,	12	16	23·51	22·31	22·92	21·17	22·89	17 children, 4 adults. 3 gas-jets for 2 hrs. Pk. sample taken as spots nearly exhausted.
15th	Consulting Room 1750 ft.	Shut up at 4.50 p.m. Spots. 5.39 p.m. Pk. 5.43 ,,	,,	15 for 10 m.	22	15·23	16·23	15·73	14·75	16·25	Two gas-jets. Gas fire. Damper partially down.
16th	,,	Shut up at 7.5 p.m. S. 7.59 p.m. Pk. 8.4 ,,	,,	18 for 10 m.	22	15·67	16·23	15·95	16·25	17·64	As before.
		S. 8.50 ,, Pk. 8.53 ,,	,,	20	25	14·11	14·28	14·20	15·75	17·10	Very windy night.
17th	Drawing Room 3200 ft.	S. 8.58 ,, Pk. 9.5 ,,	3·6	11	15	22·52	20·40	21·46	23·82	25·95	5 adults. 4 gas-jets for 1 hr.
18th	Consulting Room 1750 ft.	Shut up at 7.15 p.m. S. 8.9 p.m. Pk. 8.16 ,,	,,	9½ for 8 m.	(14)	32·99	(Ratio 29·86)	32·99	33·08	37·30	As above. Damper completely down. Spots abnormally small.
		S. 8.48 ,, Pk. 9 ,,	,,	7½ for 8 m.	(10)	41·8	(Ratio 46·06)	41·8	44·89	51·19	

19th	Consulting Room 1750 ft.	Shut up at 7.30 p.m. S. 8.19 p.m. Pk. 8.23 ,,	3·6	7	10	35·38	31·35	33·36	36·03	40	
		S. 9.9½ ,, Pk. 9.21 ,,	,,	6½	9	38·1	34·83	36·47	43·36	48·99	
21st	,,	Shut up at 7.57 p.m. S. 8.54 p.m. Pk. 8.59 ,,	,,	10	13	24·77	24·11	24·44	23·43	25·77	
		S. 9.50 ,, Pk. 9.59 ,,	,,	6	7½	41·80	41·95	41·88	40·36	45·29	
22nd	,,	Shut up at 7 p.m. S. 7.50 p.m. Pk. 7.54 ,,	5·1	8½ for 10 m.	10½ for 9 m.	33·44	33·41	33·3	30·92	34·35	Large spots.
		S. 8.35 ,, Pk. 8.38 ,,	,,	9	11	38·99	40·37	39·68	38·54	43·23	Spots usual size.
		S. 9.13 ,, Pk. 9.16 ,,	,,	7½ for 10 m.	8½	37·5	41·28	39·39	42·46	47·86	Spots large.
25th	,,	Shut up at 6.57 p.m. S. 7.50 p.m. Pk. 7.54 ,,	,,	11	(16½)	31·90	—	31·90	35·08	38·98	Small spots.
		S. 8.36 ,, Pk. 8.38 ,,	,,	9	12	38·99	37·01	38·00	37·38	41·86	Spots of ordinary size.
		S. 9.29 ,, Pk. 9.33 ,,	,,	7 for 10 m.	10	40·60	44·41	42·51	43·38	49·29	Large spots.

TABLE II. *External Air.*

Average Factor for Reduction, 74·6.

Date	Time	x = Time of exposure	S = Strength of solution in milligrammes of CO_2 per 100 c.c.	Carbonic acid in parts per 10,000: By "Spots" $\frac{S \times f}{x}$ =	Carbonic acid in parts per 10,000: By Pettenkofer unreduced	Carbonic acid in parts per 10,000: Pettenkofer reduced to 760 mm. and 0°C.	Remarks
Sept. 1904		Minutes					
17th	—	32·8	CaH_2O_2 = 1·5	3·41	3·23	3·50	
18th	—	32·2	,,	3·48	3·07	3·30	
19th	9.15 a.m.	46	CaH_2O_2 = 2·2	3·57	3·40	3·64	
	12.10 p.m.	37·7	CaH_2O_2 = 1·85	3·66	3·10	3·37	
20th	12.45 ,,	40	,,	3·45	3·12	3·52	
	4.30 ,,	39	,,	3·54	3·57	3·84	
21st	11.50 a.m.	29·6	CaH_2O_2 = 1·4	3·53	3·40	3·78	
	3 p.m.	29·4	,,	3·55	3·07	3·36	
22nd	9.30 a.m.	11½	MgH_2O_2 = ·5	3·25	3·36	3·56	
	4.30 p.m.	34½	CaH_2O_2 = 1·4	3·03	3·21	3·42	
23rd	1.15 ,,	12	MgH_2O_2 = ·5	3·11	3·08	3·36	
	2.50 ,,	34	CaH_2O_2 = 1·4	3·08	2·92	3·18	
24th	12.30 ,,				3·60	3·85	Period of low barometric pressure.
	2.20 ,,				4·16	4·43	
	3.15 ,,	$34\frac{6}{7}$	,,	3·03	3·40	3·61	
27th	9.10 a.m.	53 for 8	CaH_2O_2 = 1·8	3·17	3·69	3·92	
28th	12.25 p.m.	26 for 10	,,	4·13	3·91	4·21	
29th	2.30 ,,	29 for 10	,,	3·70	3·55	3·80	
30th	12.30 ,,	22 for 10	CaH_2O_2 = 1·5	4·05	3·64	3·94	
Oct. 1st	12.30 ,,	34	,,	3·29	3·07	3·27	
3rd	11.45 a.m.	65 for 8	MgH_2O_2 = 2	2·87	3·64	3·93	
4th	2.30 p.m.	60 for 8	,,	3·11	3·83	4·13	Period of low barometric pressure.
5th	12 m.	38 for 8	BaH_2O_2 = 1·7	4·16	4·43	4·82	

THE SEASONAL PREVALENCE OF HOFMANN'S BACILLUS[1].

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During the last six years (1899—1904) some fifteen thousand examinations of material from throats for the diphtheria bacillus have been made at the Lister Institute. The localities from which this material has been derived have naturally been widespread and varied; much of the work however has been done for local authorities in and near London, and the sources of origin of the major part of the material have not varied very much during the whole period under review.

Records have been kept throughout of the occurrence both of the genuine Klebs-Loeffler organism and of the pseudo-diphtheria bacillus of Hofmann. The diagnosis is based upon the microscopical appearances found in young (12—20 hours) cultures on serum made from the swabs sent to the Institute. The films are made from smears taken over the whole surface of the culture and no attempt is made to pick out individual colonies. These preparations have been made by the same laboratory attendant during the whole period dealt with here. In the ordinary routine, Loeffler's blue is alone used, though a certain number are also examined by Neisser's method. From time to time the accuracy of the diagnoses has been tested in a few instances by isolation of the organisms and investigation of the cultural and pathogenic pro-

[1] I have not attempted any survey of the literature of the subject: this has been recently reviewed in the exhaustive papers of G. S. Graham-Smith; this *Journal*, vol. III. p. 216, vol. IV. p. 258.

TABLE I.

	A		B		C		D	
	Klebs-Loeffler per thousand of total cases examined	Percentage above (+) or below (−) mean (282)	Klebs-Loeffler per thousand of cases without Hofmann	Percentage above (+) or below (−) mean (306)	Hofmann per thousand of total cases examined	Percentage above (+) or below (−) mean (111)	Hofmann per thousand of cases without Klebs-Loeffler	Percentage above (+) or below (−) mean (142)
January	264	− 6·4	285	− 6·9	**115**	**+ 3·6**	139	− 2·1
February	249	− 11·7	268	− 12·4	87	− 21·6	111	− 21·7
March	265	− 6·0	292	− 4·6	**118**	**+ 6·3**	**151**	**+ 6·3**
April	269	− 4·6	289	− 5·5	105	− 5·4	131	− 7·7
May	**283**	**+ 0·3**	**308**	**+ 0·6**	**126**	**+13·5**	**157**	**+10·6**
June	273	− 3·2	303	− 1·0	**128**	**+15·3**	**164**	**+15·5**
July	282	± 0·0	**307**	**+ 0·3**	**123**	**+10·8**	**154**	**+ 8·4**
August	280	− 0·7	**311**	**+ 1·6**	**144**	**+29·7**	**181**	**+27·5**
September	**289**	**+ 2·5**	**313**	**+ 2·3**	101	− 9·0	132	− 7·0
October	**321**	**+13·8**	**345**	**+12·7**	104	− 6·3	136	− 4·2
November	**303**	**+ 7·4**	**333**	**+ 8·8**	104	− 6·3	**143**	**+ 0·7**
December	273	− 3·2	290	− 5·2	88	− 20·7	110	− 22·5
Average for whole period of 72 months	282		306		111		142	

The monthly percentages which are above the mean are printed in heavy type.

perties of pure cultures. Though the necessities of time and purpose have presumably led to a certain number of mistakes, the differential diagnosis between the Klebs-Loeffler and Hofmann organisms can in general be readily made by the simple method which has been used. The morphological differences are usually obvious enough, especially when taken in conjunction with the tinctorial differentiation brought out by Loeffler's alkaline methylene blue. Strictly speaking, however, the records refer to the presence of "bacilli morphologically indistinguishable from" the Klebs-Loeffler and Hofmann organisms respectively. The actual diagnoses have been made by Prof. R. T. Hewlett, Dr Sidney Rowland, Dr A. T. MacConkey, Dr A. Moore, and a few by myself, in conjunction with Dr Allan Macfadyen.

As far as possible I have eliminated from the records those cases in which swabs were taken from persons who were merely "contacts" of diphtheria infections. The remainder comprise those who were suffering either from true diphtheria or from some affection of the throat bearing a likeness to diphtheria sufficiently close to render the bacteriological examination desirable. They number in all 14937 ; of these

4069 or 272 per thousand showed Klebs-Loeffler alone,

1521 or 102 per thousand showed Hofmann alone,

139 or 9 per thousand showed Klebs-Loeffler and Hofmann together.

So that, in all, Klebs-Loeffler was present in 282 per thousand and Hofmann in 111 per thousand of all cases examined.

In order to investigate the question of seasonal prevalence, the proportion of examinations in which each organism was found has been calculated for each month. The details are given in the Appendix ; the summarised totals are shown in Table I, and represented graphically in Fig. 1.

These show a clear difference in the seasonal variations of the frequency of positive examinations for the two organisms, *Klebs-Loeffler prevailing during September, October and November, while Hofmann is most frequent from May to August.* The curve of frequency of finding Klebs-Loeffler corresponds fairly closely with the well-known seasonal curve for the occurrence of cases of diphtheria. This points to the conclusion that the actual seasonal prevalence of Hofmann is similar to that shown in the curve of frequency of finding that bacillus in the swabs examined.

In the present series, Hofmann is much less frequently found in

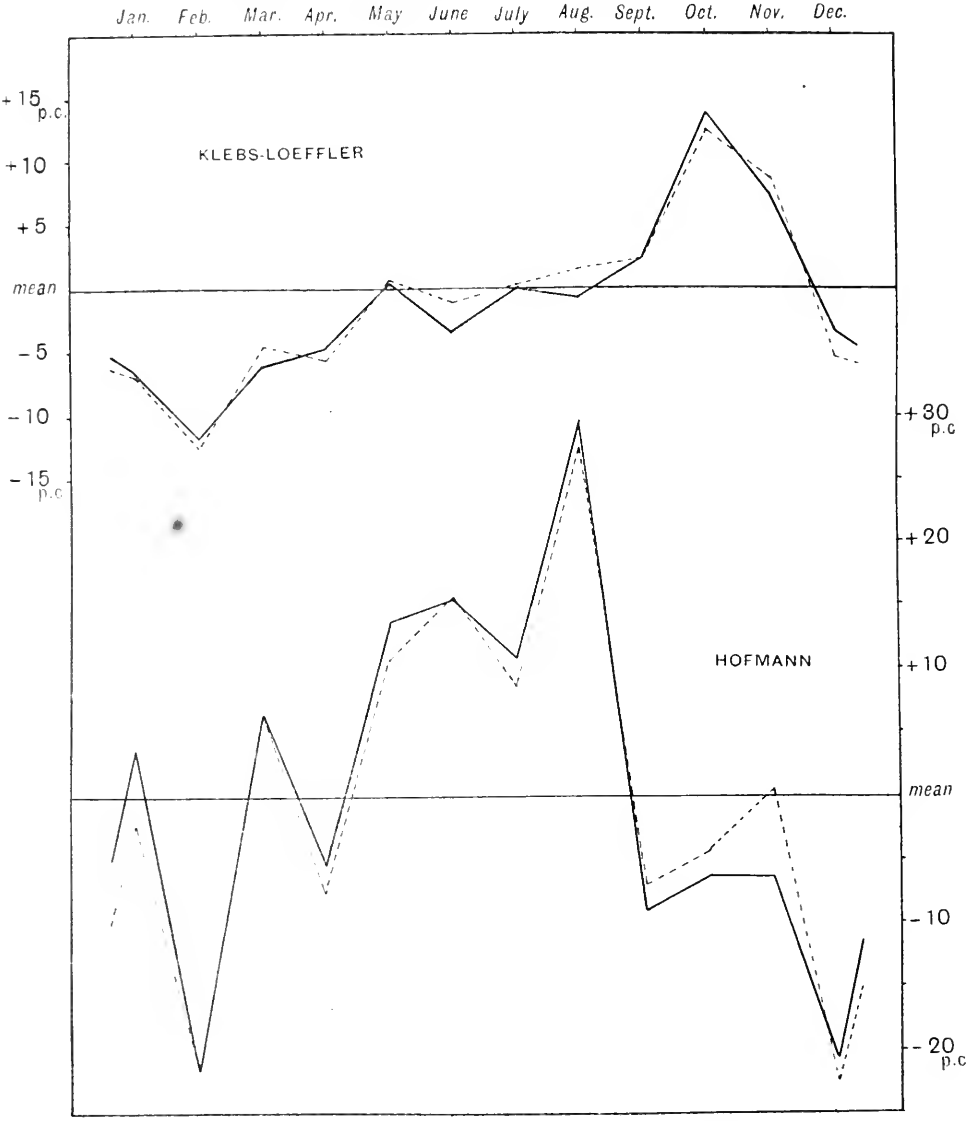

Fig. 1. The abscissae represent months; the ordinates the percentage of the mean for the whole period by which the percentage of positive examinations in each group deviates above or below the mean. The upper curves represent the proportion of Klebs-Loeffler found (1) in all cases examined (Table I, A) by the continuous line, and (2) in cases without Hofmann (Tables I, B) by the broken line.

The lower curves represent the proportion of Hofmann found (1) in all cases examined (Table I, C) by the continuous line, and (2) in cases without Klebs-Loeffler (Table I, D) by the broken line.

cases with, than in those without Klebs-Loeffler[1]. Hence any rise in the Klebs-Loeffler curve would tend to automatically lower the Hofmann curve over the same period, if both curves are based on the percentage frequency of positive examinations in all cases examined. That this factor is immaterial in the present instance is shown by the fact that the percentage deviations of the percentages of positive results for Klebs-Loeffler in cases without Hofmann, and for Hofmann in cases without Klebs-Loeffler (Figures 1 and 2, dotted curves) correspond with those for the same organism in all cases examined.

Corresponding figures for each year are given in Table II and shown graphically in Fig. 2.

As far as the percentage of all examinations which show Klebs-Loeffler is concerned, there has been a considerable fall from 1899 (336 per thousand positive) to 1903 (180 per thousand positive) with a slight recovery in 1904. There has been no corresponding decline in the number of cases of diphtheria notified in London or in England generally during the same period. The explanation would appear to be that there has been an increasing tendency to call in the aid of bacteriology on slighter grounds, and to send swabs for examination from cases which have a more remote clinical resemblance to diphtheria.

The yearly figures for Hofmann give a curve which resembles the Klebs-Loeffler curve, though the fall has been greater throughout. If the explanation given above of the fall in the Klebs-Loeffler curve is correct, the natural conclusion to draw from the similar decline in the Hofmann curve is that Hofmann's bacillus is associated with some morbid condition of the throat which resembles, but is not identical

[1] Hofmann was found in 33 per thousand of cases with, and in 142 per thousand of cases without, Klebs-Loeffler. It is probable that these figures by no means represent, at any rate quantitatively, the real frequency of co-existence. In the first place, once Klebs-Loeffler has been found in the film, further search is not always made for Hofmann. Secondly, and perhaps most cogently, if the swab is taken accurately from a definite membrane, Klebs-Loeffler may be obtained in pure culture as being the causative organism; if Hofmann has no relation to the local disease, it would probably be absent from the acute specific local lesion. It would be interesting to know how often under these circumstances it is present in other areas of the mouth, nose and pharynx. In the third place, the possibility of the overgrowth of Hofmann by Klebs-Loeffler on a medium favourable to the latter must be considered; this does not however seem to take place in artificial mixtures grown on serum.

It may be not without significance that both organisms have been found more frequently together in monthly and yearly periods which correspond more closely with the prevalence of Hofmann than with that of Klebs-Loeffler (see Appendix, Tables G and H). The cases are however very few in number.

TABLE II.

	A		B		C		D	
	Klebs-Loeffler per thousand of total cases examined	Percentage above (+) or below (−) mean (282)	Klebs-Loeffler per thousand of cases without Hofmann	Percentage above (+) or below (−) mean (306)	Hofmann per thousand of total cases examined	Percentage above (+) or below (−) mean (111)	Hofmann per thousand of cases without Klebs-Loeffler	Percentage above (+) or below (−) mean (142)
1899	**336**	**+19·1**	**377**	**+23·2**	**163**	**+46·8**	**215**	**+51·4**
1900	**316**	**+12·1**	**353**	**+15·4**	**152**	**+36·9**	**197**	**+38·7**
1901	**324**	**+14·9**	**350**	**+14·4**	99	−10·8	139	− 9·1
1902	269	− 4·6	295	− 3·6	104	− 6·3	137	− 3·5
1903	180	−36·2	191	−37·6	67	−39·6	79	−44·4
1904	231	−18·1	242	−20·9	62	−44·1	75	−47·2
Average for whole period	282		306		111		142	

The yearly percentages which are above the mean are printed in heavy type.

with, diphtheria, and that, with a more extended use of the bacteriological test, the examples of this condition have, like those of true diphtheria, been numerically diluted by an increasing proportion of relatively normal cases. If Hofmann's bacillus were a common inhabitant of the throat, giving rise to no pathological changes, it would be natural to

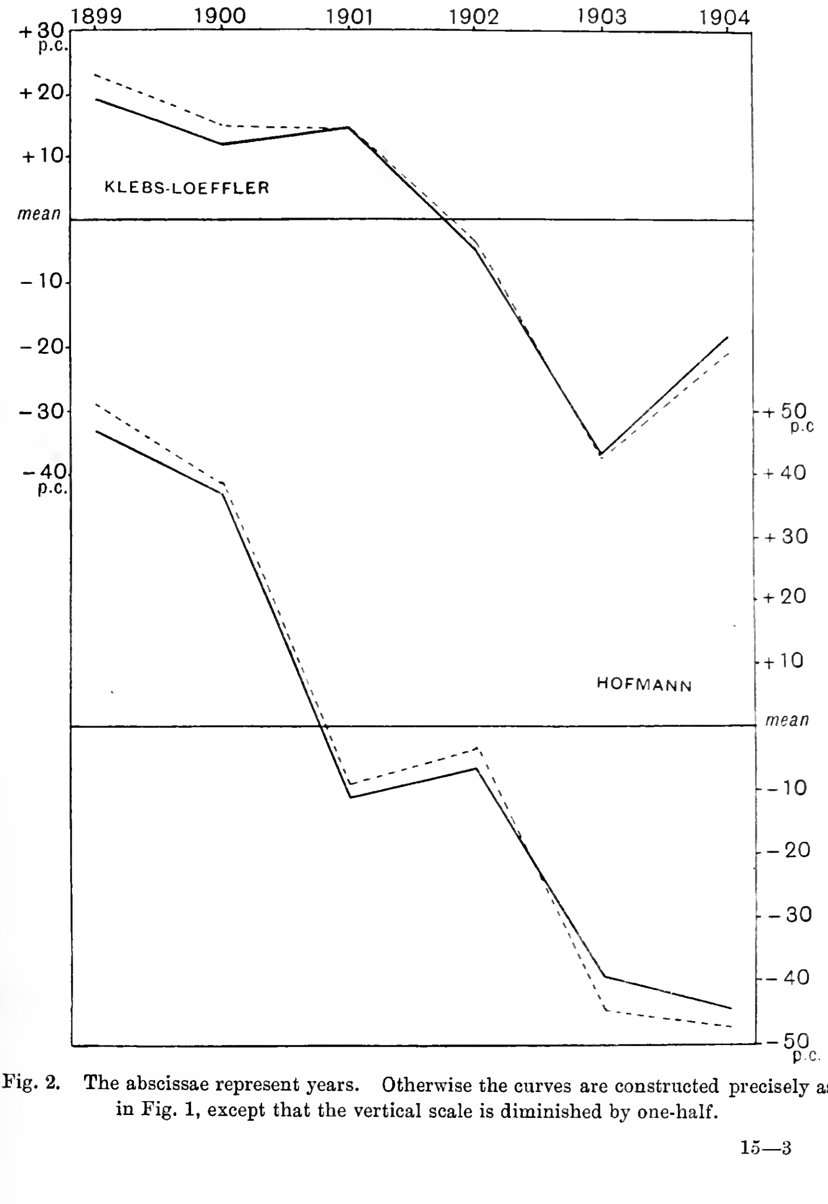

Fig. 2. The abscissae represent years. Otherwise the curves are constructed precisely as in Fig. 1, except that the vertical scale is diminished by one-half.

suppose that the frequency of its occurrence would not materially differ from year to year, or would even have risen with the addition of a larger number of cases of mild affections. The figures may, on the other hand, merely express a general diminution in the frequency of Hofmann's bacillus, irrespective of the nature of the throats examined. The view that the similarity in the yearly curves for Hofmann and Klebs-Loeffler is to be taken as evidence of an essential and close relationship between the two organisms would correspond with an interpretation of the monthly curves as showing an aestival increase of Hofmann preparatory for, and possibly causative of, the autumnal excess of Klebs-Loeffler. Such an explanation is improbable.

APPENDIX.

Table A. *Total cases examined.*

Year	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Total
1899	203	194	170	152	184	188	236	255	236	271	256	212	2557
1900	217	160	228	198	206	197	239	185	211	306	293	210	2650
1901	222	166	195	156	239	221	250	156	353	374	452	332	3116
1902	296	159	149	160	175	154	181	157	144	258	415	280	2528
1903	281	228	230	160	151	184	179	137	168	185	154	153	2210
1904	138	126	169	142	145	111	163	129	144	252	199	158	1876
Total	1357	1033	1141	968	1100	1055	1248	1019	1256	1646	1769	1345	14937

Table B. *Total cases in which Klebs-Loeffler was found alone.*

1899	75	61	53	42	59	51	76	65	90	76	95	63	806
1900	61	51	83	48	65	49	59	65	66	119	76	50	792
1901	69	39	49	50	62	66	73	34	125	135	165	116	983
1902	65	37	43	47	59	42	52	44	26	74	112	68	669
1903	49	30	36	46	34	38	43	36	21	21	18	22	394
1904	23	35	30	18	17	33	33	27	26	84	62	37	425
Total	342	253	294	251	296	279	336	271	354	509	528	356	4069

Table C. *Total cases in which Hofmann was found alone.*

1899	32	35	21	23	37	33	35	38	26	27	29	29	365
1900	46	20	38	25	27	32	26	22	25	39	47	11	358
1901	17	3	27	12	18	19	29	32	45	39	35	16	292
1902	18	12	13	11	25	19	25	20	12	27	41	30	253
1903	21	11	12	13	12	20	16	15	7	7	6	4	144
1904	5	5	16	9	5	3	7	6	3	13	19	18	109
Total	139	86	127	93	124	126	138	133	118	152	177	108	1521

Table D. *Total cases in which Klebs-Loeffler and Hofmann were found together.*

1899	6	0	0	4	5	3	7	4	5	9	4	6	53
1900	7	3	8	1	7	4	6	2	2	4	1	1	46
1901	2	1	0	2	2	0	1	6	1	2	0	0	17
1902	0	0	0	1	1	2	2	0	0	2	2	1	11
1903	2	0	0	0	0	0	0	1	1	0	0	0	4
1904	0	0	0	1	0	0	0	1	0	2	1	3	8
Total	17	4	8	9	15	9	16	14	9	19	8	11	139

TABLE E. *Cases per thousand of total cases examined in which Klebs-Loeffler was found (including Klebs-Loeffler with Hofmann).*

Year	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Total
1899	**399**	314	312	303	**348**	287	**352**	271	**402**	314	**387**	325	336
1900	313	**337**	**399**	247	**349**	269	272	**362**	**322**	**402**	263	243	316
1901	320	241	251	**333**	268	299	296	256	**347**	**366**	**365**	**349**	324
1902	219	233	**288**	**300**	**343**	**286**	**298**	**280**	180	**294**	**275**	246	269
1903	**181**	133	156	**288**	**225**	**206**	**240**	**270**	131	113	117	144	180
1904	167	**278**	177	134	117	**297**	202	217	180	**341**	**317**	**253**	231
Total	264	249	265	269	283	273	282	280	289	321	303	273	282

Percentages which are above the mean for each year are printed in heavy type.

TABLE F. *Cases per thousand of total cases examined in which Hofmann was found (including Klebs-Loeffler with Hofmann).*

1899	**187**	**180**	123	**177**	**228**	**191**	**178**	**165**	131	133	129	**165**	163
1900	**244**	144	**159**	131	**165**	**183**	134	128	128	140	**164**	57	152
1901	85	24	**138**	90	84	86	**120**	**244**	**130**	**109**	77	48	99
1902	61	75	87	75	**149**	**136**	**149**	**127**	83	**112**	104	**111**	104
1903	**82**	48	52	**81**	**79**	**109**	**89**	**116**	48	38	39	26	67
1904	36	40	**95**	**70**	34	27	43	54	21	51	**101**	**133**	62
Total	115	87	118	105	126	128	123	144	101	104	104	88	111

Percentages which are above the mean for each year are printed in heavy type.

TABLE G. *Cases per thousand of total cases examined in which Klebs-Loeffler was found with Hofmann.*

Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Total
12	4	7	9	**14**	8	**13**	**14**	7	**12**	4	8	9

1899	1900	1901	1902	1903	1904	Total
21	**17**	5	4	2	4	9

TABLE H. *Cases per thousand of cases examined with Klebs-Loeffler in which Hofmann was found.*

Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Total
47	15	26	**35**	**48**	31	**45**	**49**	25	**36**	15	30	33

1899	1900	1901	1902	1903	1904	Total
62	**55**	17	16	10	18	33

PUBLICATIONS RECEIVED.

BOOKS.

Foster, Sir C. le Neve, and Haldane, J. S. (1905), *The Investigation of Mine Air.* An account by several authors of the nature, significance, and practical methods of measurement of the impurities met with in the air of collieries and metalliferous mines. London: Charles Griffin & Co., Ltd. 191 pages. 40 figs. 3 plates. (Price, in cloth, 6*s.* net.) 20 × 14 cm.

Contains: I. A translation of the treatise on Mine Air Analysis written for mining engineers by Prof. O. Brunck of the Freiberg Mining College (pp. 1—62). II. A description of the methods of measurement of air-currents and fire-damp at the Ronchamp Collieries, by L. Poussigue (pp. 63—94). III. The examination of mine air, by Dr J. S. Haldane (pp. 95—156). IV. Appendix. The effects of carbonic oxide in connection with the Snaefell Mine Disaster in 1897. From the Report to the Home Secretary, by Clement le Neve Foster (pp. 157—188).

Keen, F. N. (1904), *Markets, Fairs, and Slaughter-houses.* London: P. S. King & Son. 78 pages. (Price 3*s.* 6*d.* net. Cloth.) 22 × 14 cm.

Contents: This book contains a "Collection of special provisions relating to markets, fairs, and slaughter-houses, contained in the Private Acts of Parliament and Provisional Orders obtained by English Provincial Corporations and Urban District Councils in the years 1901, 1902, and 1903." The book is issued in excellent form.

Keen, F. N. (1905), *Urban Police and Sanitary Legislation,* 1904. London: P. S. King & Son. 240 pages. (Price 10*s.* 6*d.* net. Cloth.) 22 × 14 cm.

Contents: The author has compiled and arranged in a very useful manner a "Collection of Provisions contained in the Local Improvement Acts obtained by English Urban District Councils in the year 1904, after consideration by the Police and Sanitary Committee of the House of Commons."

Leach, A. E. (1904), *Food Inspection and Analysis.* For the use of public analysts, health officers, sanitary chemists, and food economists. New York: J. Wiley & Sons. London: Chapman and Hall Limited. 787 pages. 278 figures. (Price 31*s.* 6*d.* net. Cloth.) 26 × 18 cm.

Contents: The book is divided into nineteen chapters devoted to:—Food analysis and State control—the laboratory and its equipment—food, its functions, proximate components, and nutritive value—general analytical methods—the microscope in food analysis—milk and milk products—flesh foods—eggs—cereals and their products, legumes, vegetables, and fruits—tea, coffee, and cocoa—spices—edible oils and fats—sugar and saccharine products—alcoholic beverages—vinegar—artificial food colours—food preservatives—artificial sweeteners—canned and bottled vegetables, relishes, and fruit products.

Appendix and an excellent Index. The work is well illustrated and printed and should prove very useful, especially to American workers. The author is analyst of the Massachusetts State Board of Health.

McCleary, G. F. (1905), *Infantile Mortality and Infants' Milk Depôts.* London: P. S. King & Son. 135 pages. 26 plates. 19 × 13 cm. Cloth.

Contents: The decline in the English birth-rate—infantile mortality—infant feeding—the "consultation de nourissons" and "goutte de lait"—the infants' milk depôt—results—objections—the cost of an infants' milk depôt.

Parts of the subject-matter have appeared in the *Journal of Hygiene*, vol. IV. The author has done well to enlarge upon various questions and to present them in such attractive form to general readers.

Mense, C. (1905), *Handbuch der Tropenkrankheiten.* Leipzig: J. A. Barth. (Price 12 Marks unbound, 13.20 Marks bound.) Vol. I. pp. 354. 124 figures and 9 plates. 25 × 16 cm.

Contents: Cutaneous Diseases of the Tropics by A. Plehn (pp. 1—74);—Diseases due to Vermes and Arthropoda by A. Looss (pp. 77—202);—Diseases of the Nervous System and Mind by P. C. J. van Brero (pp. 210—233);—Diseases due to Intoxication: (1) Intoxications through vegetable poisons by F. Rho; (2) Intoxications through animal poisons by A. Calmette (pp. 291—336).

This work is excellently illustrated and printed. The names of the collaborators on the title page sufficiently indicate that the work will prove of the first importance to those concerned with tropical medicine. If the volumes that are to follow are as thoroughly brought up to date the book will soon take its place as the principal work of reference.

Woodruff, C. E. (1905), *The effects of Tropical Light on White Men.* London and New York: Rebman Co. 358 pages. (Price, cloth, 10*s.* 6*d.* net.) 22 × 15 cm.

Contents: Zoological zones—ether waves—action of ether waves on protoplasm—difference between plants and animals—natural defences of animals from light—known effect of light in man—actino-therapy—blondness of Aryans—evolution of blondness—results of insufficient pigmentation—results of migration of blond races—results of migrations to America—practical rules for white men in the tropics—Index.

BROCHURES.

Darapsky, L. (1905), *Enteisenung von Grundwasser.* (Mit 3 Diagrammen und 5 Abbildungen.) Hygienische und gesundheitstechnische Zeitschrift *Gesundheit.* (Reprinted.) Leipzig. 104 pages.

Dudley, C. B. (1904), *The passenger car ventilation system of the Pennsylvania Railway Company.* Altoona, Pa.: The Pennsylvania Railroad Company. 24 pages. 3 figures. 22 × 16 cm.

Circular issued by the Illinois State Board of Health (1904). *The Cause and Prevention of Consumption.* Third Revised Edition. 24 pages. 1 chart. 6 plates. 25 × 15 cm.

Heisrath, F. (1904), *Ueber die Behandlung der granulösen Augenentzündung mit besonderer Berücksichtigung des Operationsverfahrens.* 46 pages. Leipzig: J. A. Barth. (Price 80 Pfennigs.) 22 × 14 cm.

REPORTS AND PERIODICALS.

BALFOUR, A. (1904), *First Report of the Wellcome Research Laboratories at the Gordon Memorial College, Khartoum.* Printed by the Department of Education, Sudan Government, Khartoum. 83 pages. Numerous figures. 6 coloured plates. 28 × 20 cm.

Contents: Description of the Laboratories—mosquito work in Khartoum and in the Anglo-Egyptian Sudan generally—biting and noxious insects other than mosquitoes—insects and vegetable parasites injurious to crops—cyanogenesis in *Sorghum vulgare*—general routine work—eosinophilia in Bilharzia Disease and Dracontiasis—mosquitoes of Egypt, the Sudan, and Abyssinia, by F. V. Theobald. (6 plates and 2 coloured figures.)

This volume is very well illustrated and printed. The contents stated above sufficiently indicate the nature of the valuable contributions it contains.

Bericht über die II. Versammlung der Tuberkulose-Aerzte Berlin, 24 bis 26 November, 1904. Herausgegeben von Oberstabsarzt a. D. Dr Nietner, Generalsekretär des Deutschen Central-Komites zur Errichtung von Heilstätten für Lungenkranke. Berlin (1905). 121 pages. 27 × 19 cm.

First Annual Report of the Henry Phipps Institute for the study, treatment, and prevention of Tuberculosis. A brief account of the work of the first year and a reprint of the lectures delivered under the auspices of the Institute during the year 1. ii. 1903 to 1. ii. 1904. Philadelphia: published by the Henry Phipps Institute. 265 pages. 11 plates. Several charts and a map. 25 × 17 cm.

Fourth Annual Report of the New York State Hospital for the Care of Crippled and Deformed Children, for the year ending September 30, 1904. Hospital located at Tarrytown, N. Y. Albany, U. S. A. 28 pages. 6 plates. 23 × 15 cm.

Journal of Experimental Medicine, Vol. VII. No. 1, issued 25. ii. 1905. Edited by Simon Flexner and E. Opie, and published under the auspices of the Rockefeller Institute for Medical Research, New York. The Macmillan Co., New York and London.

Contains: Dawson, P. M., The changes in the Heart Rate and Blood "Pressures" resulting from Severe Haemorrhage and Subsequent Infusion of Sodium Bicarbonate (pp. 1—31). Emerson, H., and Norris, C., "Red-Leg"—an Infectious Disease of Frogs (pp. 32—58). Meltzer, S. J., and Auer, J., On the Rate of Absorption from Intramuscular Tissue (pp. 59—78). Plates I.—III. Herter, C. A., The Color Reactions of Naphthaquinone, Sodium-Monosulphonate and some of their Biological Applications (pp. 79—110). Taylor, A. E., On the Preparation of Salt-Free Culture Media and the Growth of Bacteria upon them (pp. 111—118).

We welcome the reappearance of this first-class Journal, the editorship of which has been resigned by its founder Prof. Wm. H. Welch of Baltimore.

Publications from the Laboratories of the Jefferson Medical College Hospital. Philadelphia. Vol. I. (1904). Reprints bound. 24 × 16 cm.

REYNOLDS, A. R. (Commissioner) (1903), *Vital Statistics of the City of Chicago for the years* 1899—1903 *inclusive.* Department of Health, City of Chicago. 128 pages. Cloth. 22 × 15 cm.

REPRINTS.

Allen, H. B. (20. ix. 1904), *Syphilis, with reference to Hereditament, Diagnosis and Prevalence.* Intercolonial Med. Journ. of Australasia, Melbourne. Reprint. 26 pages. 25 × 16 cm.

Christophers, S. R. (1904), *On a parasite found in persons suffering from enlargement of the Spleen in India.* Second Report. N. S. Scientific Memoirs by Officers Med. and Sanit. Departments of the Government. India, Calcutta. No. 11. 21 pages. 2 plates. (Price 3*s.*) 30 × 24 cm.

Higgins, C. H. (1904), *Actinobacillosis.* Dom. of Canada. Department of Agric. Health of Animals Branch, Biol. Lab. Bull. No. 1. 6 pages. 8 plates. Ottawa. 25 × 17 cm.

Merrill, E. D. (1. x. 1904), *New or Noteworthy Philippine Plants, II.* Department Interior, Bureau of Government Labs., Manila. No. 17. 47 pages. 3 plates. 23 × 15 cm.

Musgrave, W. E. (1904), Part II. *Treatment of Intestinal Amebiasis* (Amebic Dysentery) in the tropics. Department Interior, Bureau of Government Labs. Biological Lab. No. 18, pp. 91—117. 23 × 15 cm. (Manila: Bureau of Public Printing.)

Musgrave, W. E. and Clegg, M. T. (x. 1904), Part I. *Amebas: their Cultivation and Etiologic Significance.* Department Interior, Bureau Government Labs. Biological Lab. No. 18. 85 pages. 32 figures. 23 × 15 cm. (Manila: Bureau of Public Printing.)

Strong, R. P. (ix. 1904), *Protective Inoculation against Asiatic Cholera (an Experimental Study).* Department Interior, Bureau of Government Labs. Biological Lab., Manila. No. 16. 52 pages. 14 tables. 23 × 15 cm.

VOLUME V JULY, 1905 No. 3

CANINE PIROPLASMOSIS. II.

BY GEORGE H. F. NUTTALL, M.A., M.D., Ph.D., F.R.S.,
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AND G. S. GRAHAM-SMITH, M.A., M.B.,
John Lucas Walker Student.

(*From the Pathological Laboratory, Cambridge.*)

IN a previous paper[1] one of us has summarized what is known with regard to canine piroplasmosis, and presented the results of infection experiments carried on in Cambridge with infected ticks (adult *Haemaphysalis leachi* Andouin) imported from South Africa. In the present paper we propose to describe and figure the parasite as observed in stained preparations, and to state what is known regarding its biology. The supply of infected ticks having unfortunately been exhausted and our last attempt at the transmission of the disease by infected blood inoculation having failed, we are obliged, for the present, to postpone a detailed description of the living parasite.

Piroplasma canis.

The history of the discovery of the parasite has been given in the paper above referred to (p. 223), nevertheless there are still a number of interesting facts to be considered which are stated in papers by other authors[2]. It appears advisable for this reason to briefly summarize what is known with regard to the parasite.

[1] Nuttall, G. H. F. (IV. 1904). Canine Piroplasmosis, I., *Journ. of Hygiene*, IV. pp. 219—257, Plates XII—XIII, 8 Temperature Charts.

[2] The papers here cited are given in the bibliography accompanying the previous paper (pp. 250—252).

The position of the Piroplasmata among the Protozoa has not as yet been satisfactorily determined. The generic name Piroplasma was given to them for the reason that they frequently are pear-shaped. They are endoglobular parasites, growing and multiplying within the red blood corpuscles. They do not produce melanin granules. The parasites are also encountered in a free state in the plasma. The existence of amoeboid forms was first noted and figured by Piana and Galli-Valerio (1895 and 1896), and has been repeatedly observed since by various workers. Irregular, spherical, and typical pear-shaped parasites have been also observed in the disease as studied in different countries. It is premature to assume that the *Piroplasma canis* of South Africa is identical with that of Europe, or India, nevertheless there is at present no proof that they are different species.

Piroplasma canis in Italy is supposed to be conveyed by *Ixodes reduvius.* It is found in 3—4 °/₀ of red blood corpuscles; free forms also occur. The parasites measure 3·5—2·5 μ. Piana and Galli-Valerio stated that the corpuscles contained 2—5 pyriform parasites.

Piroplasma canis in France is supposed to be conveyed by *Dermacentor reticulatus.* The parasites (Almy, 1901, p. 375) are found in a variable percentage of red blood corpuscles, and also as free forms. They measure 2—4 μ. At the beginning of the disease only single large, round parasites are found in the infected corpuscles, later from 2—16 occur in the corpuscles in the acute cases. Under these conditions the parasites are small, irregular, and seldom pyriform (Nocard and Motas, 1902, p. 265). Many parasites, mostly small and spherical, occur in the heart and internal organs (*Ibid.* p. 269). Groups of 10—12, apparently free, parasites seem to lie in spaces which are probably the remains of corpuscles (Marchoux, 1900).

Piroplasma canis in South Africa is certainly conveyed by *Haemaphysalis leachi* Andouin in the adult stage (see the previous paper). Its essential characters are similar to those of the parasite observed in Europe. This is the parasite which we describe in the following pages.

Time when the Parasites appear in the Blood after experimental Infection.

According to Nocard and Motas (1902, p. 273) infected corpuscles are rarely encountered within 36 hours after inoculation. Even after intravenous inoculation they usually do not appear until the end of 48 hours.

Robertson (1902 a, p. 331) in South Africa did not find the parasites

microscopically in the blood before the fourth day after subcutaneous inoculation. That they were present earlier was proved by the fact that blood taken from a dog three days after inoculation proved infective. The parasites were present singly or in pairs. In our experiments the parasites, whether infection was produced by means of ticks or by blood inoculations, made their appearance in appreciable numbers immediately before the onset of fever.

Persistence of the Parasites in the Blood.

We found that the number of parasites present in the peripheral blood varied considerably, irrespective of the attacks of fever. From this it appears reasonable to conclude that the multiplication may be taking place in the internal organs when few parasites are present in the peripheral blood. The persistence of the parasites in the blood of "salted" dogs (animals which have recovered), has already been referred to in the previous paper under the heading of Immunity (p. 246). We have had the opportunity of studying acute cases only, in which the parasites are moderately or very plentiful. Nocard and Motas (1902, p. 265) state that the parasites are scarce in chronic, as compared with acute cases.

Structure of the Parasite.

Stained by Leishman's method, the parasite appears as a blue-staining body containing usually a single homogeneous nucleus (karyosome) which stains a vivid red. The blue-staining protoplasm frequently presents a delicate vacuolated or trabecular structure, and is chiefly condensed about the periphery. These appearances are especially marked in the larger forms. Young spherical parasites often appear as "rings" resembling young malarial parasites in man, in which the pink colour of the blood corpuscle may be seen in the central clear space within the blue-staining ring. In dividing forms a delicate protoplasmic thread may persist for some time as a connecting link between daughter cells as shown in Figs. 12, 15, 28, 55, 63, and 64. Where paired pyriform parasites occur this connecting thread is as a rule situated at their pointed extremities. In other cases the connecting threads are somewhat irregularly distributed, as in Figs. 25, 26, and 41. In the latter figure and in Fig. 42 the parasites appear to be amoeboid. Occasionally (as shown in Figs. 24, 43, and more especially Fig. 40)

apparently detached masses of blue-staining protoplasm are encountered in the corpuscle. In some cases these appeared to be connected by very delicate faint threads to each other and the parasite. In forms which appear to be the amoeboid (Plate IX, Figs. 35—36) portions of the protoplasm stain a more intense blue than do others. At times the processes may appear fragmented as in Fig. 35. The chromatin mass is usually spherical and is generally situated in the centre of the main mass of protoplasm, although frequently placed excentrically and at times peripherally. In some cases it appears to protrude from the protoplasm (Figs. 1—2, 35—39). The chromatin mass is frequently surrounded by an unstained halo, representing what appears to be a vesicular nucleus (Figs. 1, 13, 19, etc.). In dividing forms the chromatin mass assumes an elongated form before breaking up into two portions (Figs. 2, 3, 11, 57), and this separation of the nuclear masses immediately precedes the division of the protoplasm (Figs. 2, 4, 5, 6, 10, 12, 16, 18, 50—54). When about to divide the chromatin mass frequently takes up a peripheral position (Figs. 2, 3, 9, 11). The smallest chromatin masses measured about ·2 μ, the largest ·6—·8 μ in length, in the same specimen. Undoubtedly the size of these masses varies according to the method of staining employed.

Resemblance of the Parasite to other Piroplasmata.

It is obvious from the appearance of the canine parasite that it offers a great resemblance to other Piroplasmata, notably that of Texas fever (*P. bigeminum*) in cattle and that of Redwater in sheep (*P. ovis*). According to Marchoux (27. I. 1900) *P. canis* in France is larger than *P. bigeminum*, and differs in being less constantly bigeminate and in the fact that single parasites occur more frequently. In the former disease corpuscles containing 10—12 parasites may occur, as well as extra-cellular forms in pairs or in groups up to 8—10 individuals. On the other hand Piana and Galli-Valerio (1895), who first discovered the parasite in Italy, found it to resemble the Texas fever parasite so closely that they named it *Pyrosoma bigeminum*, var. *canis*, and Nocard and Motas (1902, p. 275) say that the French parasite is morphologically identical with that of Texas fever. The resemblance of the South African parasite to *P. bigeminum* is also well-marked, but Robertson (1902, a, p. 331) states that it is larger, and is more oat-shaped than pyriform. From specimens, which one of us has studied, the dog parasite does appear to differ from the Texas fever organism as stated by Robertson.

Size of the Parasites.

The canine parasites observed in Italy by Piana and Galli-Valerio (1895) measured 2·5—3·5 μ in their largest diameter, those observed by Marchoux (1900) in France 2—4 μ. Nocard and Motas (1902, p. 269) state that the parasites are larger in young dogs, which are more susceptible and may fill as much as half of the corpuscle. In adult dogs near the end of the disease they may be very small, being almost reduced to a nucleus.

Our measurements show the African parasite to be somewhat smaller. The smallest specimens measure from ·7 to 1·2 μ, the largest about 3·6 μ. Rarely pyriform bodies measuring up to 4·5—5 μ were encountered. The variations in size are however considerable, and are best seen by reference to Plate IX, where the red blood corpuscles of normal size (Figs. 1—12, 58 etc.), measuring on an average 7·2 μ, may be used as a standard for comparison. The large gamete-like bodies (Figs. 58—62) not hitherto described by other authors, measured 10·3—10·7 μ in length by 1·4—1·7 in width in their greatest diameter. All our measurements were made on stained preparations.

The above measurements (excepting the last) may be compared with those given for *P. bigeminum*. Smith and Kilborne (1893) give the size of the smallest parasites as 0·5 μ. Laveran and Nicolle (1899) working about Constantinople found the smaller parasites measured 1—2·5 μ, the largest up to 3·5 by ·8—1·2 across the base. Ziemann (1902) in Venezuela found small parasites measuring ·75—1 μ, and larger ones up to 2·5—3 μ. The measurements for *P. ovis* show that the parasites are about the same size. Bonome (1895) in Italy for example found parasites measuring from 1—3 μ up to 2—2·5 μ, and Laveran and Nicolle (1899) give the measurements for the parasites observed in sheep near Constantinople at 1—1·5, and occasionally 2 μ (in the spleen).

Parasites viewed in Fresh Blood.

Where the parasites are not numerous, they are difficult to find in fresh films. Nocard and Motas (1902, p. 265) state that they are best observed immediately after the fall of fever, the only time when they are numerous and motile. Examination is facilitated by diluting the blood with aqueous humour or saline solution in a hanging drop and using the warm stage. The infected corpuscles appear somewhat enlarged and pale, the parasites are irregular and have a dark contour, with a

central refractive portion. The French observers saw amoeboid bodies with processes, sometimes 2 or 3 in number, protruded toward the periphery of the corpuscle. Rapid movement caused the corpuscle to revolve on its axis. At times the parasite appears globular, and non-motile, and lies centrally in the corpuscle, and small, very actively moving bodies are seen dancing about it. They state that amoeboid motion quickly ceases after the febrile stage, the parasites then remaining spherical and quiescent. Free parasites are difficult to distinguish from cellular detritus, and are best seen in saline tinted faintly with methylene blue. This method is also an aid in the examination of intracellular parasites, as the Piroplasmata become faintly stained without being killed. These observations naturally explain the variations in form observed in stained blood films.

In our dogs, infected with the South African form of the disease, parasites were observed a few times in fresh blood films, but unfortunately the parasites were very scarce on these occasions, and we did not pursue the matter further at the time. We have since lost the strain, but we hope to make further investigation on this point.

Multiplication of P. canis.

It is obvious that one mode of multiplication is by direct fission, in the manner represented in the successive Figures 1—8, and 9—32 in Plate IX. The parasite, having penetrated the corpuscle, grows in size and then successively divides (usually) into 2, 4, 8, and 16 individuals. Owing to irregularities in division, groups of 3, 5, 6, 7, 9, 10, 12, 14, 15, 18, and even 21 may be encountered. Division forms giving multiples of 2 (2—16) are by far the most numerous, and the usual number reached before the corpuscle ruptures and liberates its contents, appears to be 16. The corpuscles containing numerous parasites, 8—16 or more, are more frequently encountered in the blood from the internal organs, notably the brain, lymphatic glands, bone-marrow, etc., and it is doubtless due to their accumulation in the brain capillaries that the animals become comatose in a manner similar to that which has been observed in severe cases of human malaria[1]. Robertson (1902, a, p. 332) has already noted this blocking of the cerebral capillaries by infected

[1] Bowhill gives two photomicrographs of *P. canis* on Plate III, Figs. 11—12 of this volume. Fig. 11 shows a group of parasite-containing corpuscles in a kidney capillary, Fig. 12 eight parasites with large chromatic masses lying in a corpuscle. See also Vol. IV, Plate XI.

corpuscles. In smears from the internal organs groups of 8—16 or more parasites are frequently encountered, sometimes embedded in the faintly-staining detritus of a ruptured corpuscle, while in other cases all traces of corpuscular substance have disappeared. A group of free parasites is shown in Figure 46. The parasites decrease in size as their number increases within the infected corpuscle. The small free parasites correspond in size with the youngest forms encountered in corpuscles. From this we may conclude that the parasites which escape from ruptured corpuscles again attack and penetrate new corpuscles. In one case we observed two parasites lying in a nucleated red blood cell (Fig. 45).

Most authors are agreed that this is a mode of multiplication in other Piroplasmata. Other modes of multiplication are unknown. Marchoux (27, I. 1900) working with the French parasite represents multiplication by fission of single spherical forms into 2—4—6—12 pyriform parasites, when the corpuscle ruptures and sets them free. He states that single parasites in corpuscles are usually round or oval, pairs of parasites usually pyriform. Multiplication along the lines indicated has also been described by Nocard and Motas (1902, p. 269), who figure the French parasites. They state that multiplication is most active during fever periods and that it is not well observed in the peripheral blood, where, they assume, it takes place too rapidly and irregularly. They figure two series of multiplication forms from the blood and internal organs respectively, which are in general agreement with our figures above described. According to these authors our Figure 47, with its central spherical nucleus, represents a parasite at rest. They consider that the nucleus next elongates and moves to the periphery, where it divides as we have shown in Fig. 2; that the nuclei next move to opposite poles as in Fig. 5 and fission takes place as in Fig. 6. They give some figures in a series of drawings they made from peripheral blood showing curved rod-like masses of chromatin lying close to the periphery of the parasites. We have failed to observe such forms. On the other hand they do not figure dividing pyriform parasites such as we represent in Figs. 9, 10, 11, 53, 54. Their reason for stating that the globular forms represent "l'état normal du parasite" does not appear quite clear to us. They observed 1, 2, 4, 8, 16, and 18 parasites in infected corpuscles, and 3, 6, 12, and 14 if one failed to develop. In the vast majority of cases they noted parasites in even numbers. They found kidney smears to show the greatest number of corpuscles containing numerous (12—14—16—18) parasites. During

the febrile stage of the disease they encountered round parasites with elongated excentric nuclei, measuring $\frac{1}{5}$ the length of the margin of the parasites—these represent the rapidly multiplying forms above noted. According to these authors the most active multiplication, judged by the number of infected corpuscles observed, takes place in the following order; kidney, spleen, liver, marrow, lung, heart, lymphatic glands, intestinal mucosa, central nervous system. Robertson (1902, a, p. 331) in South Africa also noted the great number of parasites in smear preparations made from the liver and spleen.

Free Parasites.

We have noted that the parasites escape into the plasma from the disintegrated corpuscles and that they may at times be found in groups similar to those seen in corpuscles. These groups soon break up, and then the parasites are found singly or in coherent pairs as typical bigeminate pyriform or ovoid bodies. Very small ovoid or spherical bodies are also encountered in a free state (Figs. 46—58). Robertson (1902, a, p. 331) especially noted free parasites in the blood in advanced stages of the disease. He gives rough sketches of very small size in which the free parasites appear to be of variable shape and size, all single, spherical, irregular or pyriform. Nocard and Motas (1902, p. 269) note the fact that free parasites may appear somewhat larger than the endoglobular forms. We have made similar observations.

Sexual Forms?

In Volume IV of this *Journal* (Plate XI) Bowhill and Le Doux stated that they observed what appeared to be flagellate bodies in the blood of dogs suffering from Piroplasmosis in South Africa. We have not as yet observed such forms which are similar to bodies noted by Lignières (1900) in Piroplasmosis in cattle in Buenos Aires. Bowhill has since described in the current volume of this *Journal* (p. 16, and Plates I—III), similar bodies in connection with *Piroplasma equi*, and *Piroplasma bigeminum* of South African Redwater. Possibly these bodies represent microgametes.

On the other hand we have observed a body which is so strikingly suggestive of a gamete that we especially draw attention to it. These bodies were encountered in the blood of the first dog experimentally infected in Cambridge, being found on the 4th, 5th, and 10th days after

the onset of fever. These bodies which somewhat resemble the crescents of the human aestivo-autumnal malarial parasites are shown on Plate IX, Figs. 58—62. Only four have been encountered in the peripheral blood after prolonged search in stained films. Three others have been seen in smear preparations from the organs, one in a lung smear, and two in kidney smears. These bodies are sausage shaped, of fairly uniform width, and rounded, or tapering at their extremities. Some are markedly vacuolated. In these cases, when the vacuoles happen to lie upon red blood corpuscles (Fig. 58) the contour and colour of the corpuscle can be seen through the vacuole. In three out of the four specimens figured the chromatin is almost entirely concentrated in the middle of the body, and in two appears to be of loose texture. In Fig. 58 particles of chromatin of fairly uniform size are distributed about irregularly, mostly near the periphery. These bodies measure 10·4 to 10·7 μ in length by 1·4 to 1·7 μ in width. They are faithfully reproduced in the figures as they appeared in films stained by Leishman's method. These bodies certainly merit further investigation. It is at present impossible to say if they stand in any relation to the bodies shown in Figs. 43—44, the latter of which is very peculiar. Nocard and Motas figure bodies somewhat like Fig. 43 showing 1—4 fine blue-staining processes, evidently amoeboid bodies.

Behaviour of the Parasites outside the Body.

Working with the French parasite Nocard and Motas (IV. 1902, p. 274) found that it remained alive and virulent in blood preserved in the dark and cold (during winter) for 25 days. Blood kept in the dark at summer temperature lost its virulence after 14 days. Blood heated to 43° C. for 90 minutes was still virulent, but became non-virulent after being heated to 44° C. for 75 minutes, or 45° C. for 60 minutes, or 50° C. for 30 minutes. They state that heating to 45° C. or above for an hour (p. 287), renders the blood non-virulent, whilst heating to 44° C. for the same time lowers its virulence slightly. Animals inoculated with the latter die more slowly than usual. Heating the blood to 44° C. for 75 to 90 minutes rendered it non-virulent.

Attempts at cultivation (p. 275) have failed on media composed of defibrinated dog's blood, serum rich in haemoglobin, and blood rendered incoagulable by the addition of leech-extract. Leeches which had fed on highly infected dog's blood have been kept at 22° C., but after a week only the nuclei of the parasites could be detected.

Observations on defibrinated blood kept at 37° C. showed that the infected corpuscles were taken up by leucocytes. The parasites became spherical, their nuclei took up a central position, and their protoplasm appeared to gradually shrivel away and set free the nuclei. Similar changes occurred in blood kept at 22° C. but more slowly, so that a little protoplasm still remained surrounding the nuclei after 5—6 days. Nuclei resembling micrococci were alone found within degenerated corpuscles after some weeks.

Robertson (5. IV. 1902, p. 685), working with the South African parasites, states that he unsuccessfully tried in many ways to preserve blood in a virulent condition outside the body. He found that such blood either killed or did not kill, but in the latter case did not confer immunity. One of us successfully infected a dog with blood which had been kept in ice for 24 hours in the dark. Inoculations with older blood were not tried. Similar changes to those described by Nocard and Motas were observed in stored blood.

Changes in the Red Blood Corpuscles.

The infected corpuscles do not alter much in size. Those containing 1—6 parasites are about the same size as normal corpuscles, namely 7·1 μ. Corpuscles containing 7—10 parasites measure on an average 8 μ, while those containing 11—16 parasites measure 9 μ. These figures are based on a large number of measurements, the mean being taken in cases where the corpuscles were altered in form. Corpuscles containing 12 or more parasites, as is indicated in Plate IX, Figs. 26—31, show a tendency to stain more feebly and to lose their round contour. As the disease progresses, nucleated red cells appear occasionally in the peripheral blood, and pale-staining, often enlarged corpuscles are encountered. In fresh blood these corpuscles may at times be almost invisible owing to their containing little haemoglobin. That the infected corpuscles soon begin to undergo some degenerative change is indicated by the fact that they are taken up by leucocytes upon which they must exert a positive chemiotactic action, possibly by the diffusion of their substance into the plasma. The changes in the blood count observed by Nocard and Motas in the French disease have already been referred to in the previous paper (Vol. IV, p. 238) and have been studied by Dr Wright in connection with our work. The relative number of infected corpuscles found under different circumstances will be treated of in Part IV.

Leucocytes.

In Figs. 63—67, Plate IX, infected corpuscles are depicted within leucocytes, together with the progressive changes the former undergo. It will be seen that the corpuscles stain a pale yellowish colour and shrink in size, the parasites they contain also degenerate and no longer show the characteristic blue-staining protoplasm (Fig. 65). Later they cease to take up the blue (Fig. 66), and finally all traces of them except the resistant chromatin masses, which show out clearly in the blue-staining protoplasm of the leucocyte, are lost (Fig. 67).

Staining Method employed.

Piana and Galli-Valerio (1895 and 1896) in Italy stained blood films by means of thymolmethylene blue, the corpuscles appearing pale blue, and the parasites dark blue, with an unstained spot representing the vesicular nucleus. Their appearance is figured in a coloured plate. Marchoux (1900), in France, used Laveran's stain (*Compt. rend. Soc. de Biol.* 15. IV. 1899) which stains the chromatin violet-red. He described the nucleus as round or elongated, and situated peripherally (its position not being as constant as in the parasites of Texas fever), lying between the margin of the parasite and a line of blue-staining protoplasm which borders the central unstained part. Nocard and Motas (1902, p. 265) stained by means of Nicolle's carbol-thionin. They dried the film in the air, fixed it in alcohol-ether or absolute alcohol, stained for 30 seconds, washed, dried, and mounted in balsam. With this stain the corpuscles appear pale green, the parasites dark blue and sharply defined, their central portion remaining unstained or staining a pale blue. They also used a modification (pp. 266—267) of Laveran's stain.

Robertson (1902 a, p. 331), in South Africa, recommends carbol-thionin blue, methylene blue, and the double stain of Plehn and Czinzinski. He noted that the parasites stained more deeply at places about the margin, and that there was in the centre an unstained area.

We obtained such excellent results with Leishman's modification of the Romanowski stain that we have employed this method throughout. This well-known stain colours the red cells yellowish-pink or pink. The corpuscles usually appear paler (see Plate IX) when occupied by numerous parasites, and more yellow when included in leucocytes within

which they progressively shrink and degenerate (Plate IX, Figs. 63—67). The protoplasm of the leucocytes appears blue, and the nuclei of the leucocyte and nucleated red corpuscles appear violet.

Further Note on the Pathogenicity of P. canis.

On page 245, Vol. IV, of this *Journal*, the results were recorded of inoculation experiments of different animals made by Robertson in South Africa, and by Nocard and Motas in France. These experimenters only succeeded in infecting dogs with *Piroplasma canis.* With a view to further testing the question we inoculated cats, ferrets, hedgehogs, guinea-pigs and white rats, two examples of each species being employed. The results were all negative. We had hoped to experiment with other Carnivora, especially foxes and other Canidae, but were unable to obtain them. It would be of interest to determine what other animals besides dogs are susceptible to infection with this parasite.

Piroplasma-like Parasite of the Mole.

Whilst investigating blood films prepared from a variety of animals from the vicinity of Cambridge, one of us discovered a piroplasma-like parasite in the mole. We wish but to record the fact, and to state that the new parasite will be described in the next number of this *Journal.*

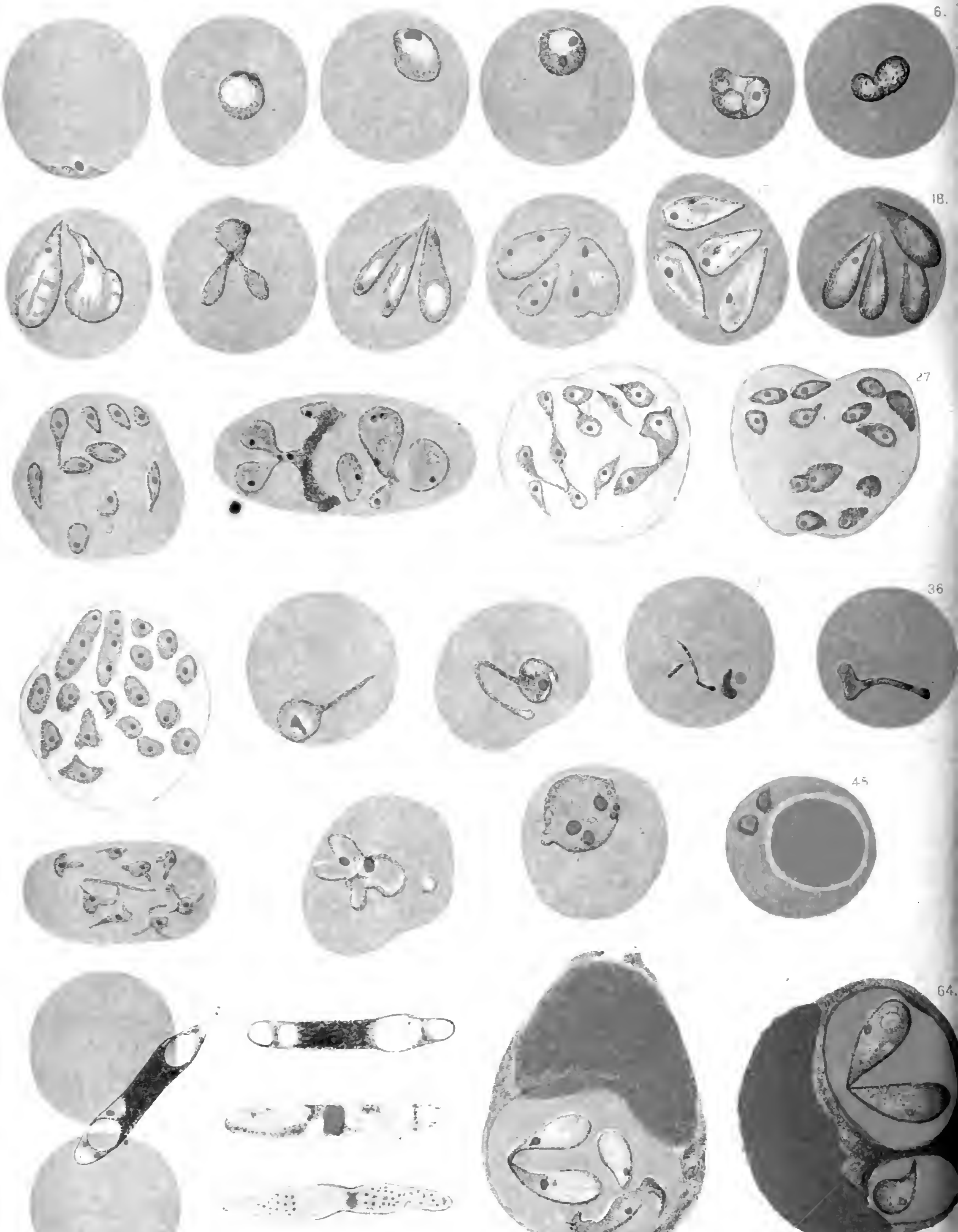
EXPLANATION OF PLATE IX.

Piroplasma canis in the blood of dogs experimentally infected in Cambridge with the South African disease. The dogs were infected by ticks and by blood inoculations. Stained by Leishman's method. Drawn with Zeiss Apochr. $\frac{1}{12}$ oil immersion objective and eyepiece 12, larger than they were seen because of the impossibility of reproducing the details in smaller figures. The figures in the Plate consequently all appear magnified about 3400. Drawn by G. H. F. N.

Figs. 1—8. Young intracorpuscular forms, rings, and dividing ovoid bodies ending in a group of four. Blood obtained chiefly from the peripheral circulation, also from spleen and lung smears.

Figs. 9—32. Series of intracorpuscular parasites beginning with a large single oval parasite. Longitudinal fission leading to groups of typical pyriform bodies etc., in groups of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 21. The nuclear division is seen to precede the separation of the daughter cells. Figs. 9 and 10 from liver and spleen smears; 12—15, from peripheral blood, the rest chiefly from spleen, bone-marrow, lung, and liver smears.

Figs. 33—42. Amoeboid forms in spleen and marrow smears.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18.
27
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64.

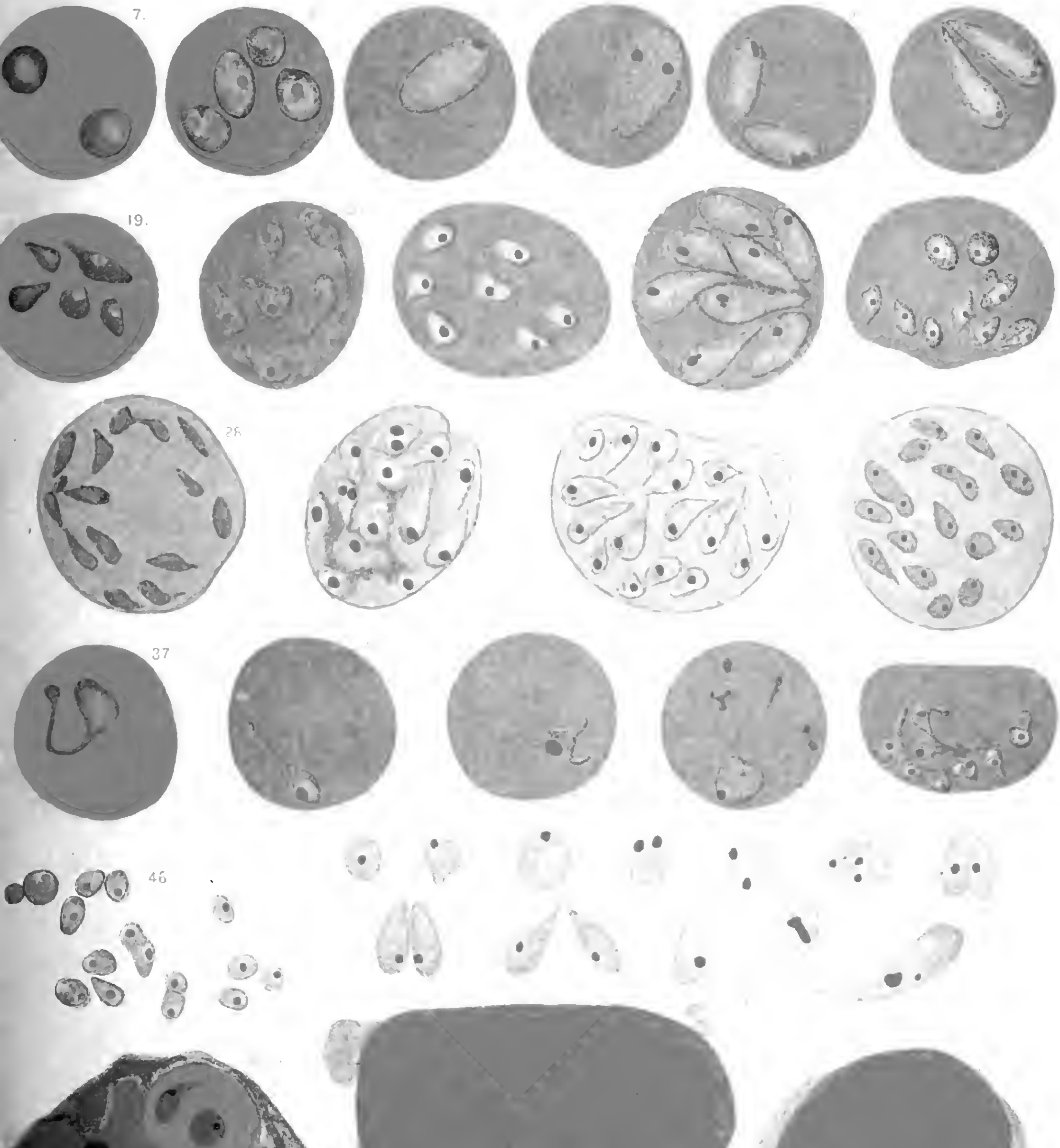
7.
19.
28
37
46

Fig. 43. Large amoeboid form in peripheral blood.

Fig. 44. Large spherical form with exceptionally large chromatin masses. Developing sexual form? (brain).

Fig. 45. Nucleated red corpuscle containing two parasites.

Fig. 46. Group of 16 free parasites liberated from a corpuscle of which all trace has disappeared (lung).

Figs. 47—58. Parasites in various stages of development and lying free in the plasma (lung and spleen).

Figs. 59—62. Sausage-shaped gametes (?) lying free in the plasma in peripheral blood.

Figs. 63—67. Infected corpuscles taken up by leucocytes and undergoing progressive degeneration in order of the figures from left to right. In Fig. 67 only the chromatin masses of the parasites are left.

CANINE PIROPLASMOSIS. III.

MORBID ANATOMY.

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CONTENTS.

In the preceding paper the morphology of the parasites found in the peripheral circulation and in the organs of eleven dogs experimentally infected with Canine Piroplasmosis has been described. In the following pages will be considered the morbid lesions found in these dogs, and the proportions of the variously infected red corpuscles to one another, both in the organs and in the blood.

Dogs Nos. I and IV were given the disease by means of infected ticks, the others by the subcutaneous inoculation of blood from other infected dogs. All these dogs suffered from the acute, or subacute,

form of the disease, the duration of the disease varying between 13 (Dogs V and VI, puppies) and 47 days (Dog VIII).

Parasites in the peripheral circulation. In the majority of cases blood films were made daily from the date of infection to the day of death. The films were stained by Leishman's method, and thoroughly examined for the presence of parasites and nucleated red cells, and at the same time records were made of the proportion of corpuscles infected with one or more parasites.

Parasites were never seen in the films until several days after infection. The earliest date on which they appeared was the 6th day (Dog XI), and the latest the 36th day (Dog VIII). In most of the other animals they were first seen between the 8th and 12th days after infection.

Percentage of infected cells. The proportion of infected to non-infected red cells was only worked out in a few instances.

In Dog I from their first appearance until the day before death, the percentage of infected to non-infected red blood corpuscles varied between ·3 and ·6 %. On the day before death it rose to 1·4 %. At the autopsy 1 % of the blood corpuscles from the heart were found to be infected.

In Dog II only ·05 % of the red blood corpuscles were infected 5 days before death. Two days later ·3 % were infected. On the day preceding death there were ·8 % of infected corpuscles and on the day of death 2·8 %.

TABLE I. *Showing the percentage of infected corpuscles and the duration of the disease.*

	Percentage of infected corpuscles		
	Day before death	Autopsy	Duration of the disease
Dog VI	6·0 %	4·0 %	13 days
VII	4·0	·5	32
I	1·4	1·0	25
X	1·4	2·0	23
II	·8	2·8	23
XI	·5	1·2	17
III	·4	·8	22
IX	·3	·7	24
VIII	·2	·4	47
IV	·1	·4	23
V	?	·05	13

TABLE II. *Showing the numbers of parasites found in infected corpuscles in the peripheral circulation and heart's blood in eleven dogs.*

Number of parasites within infected corpuscles	Two days and more before death: No. of infected corpuscles counted	Two days and more before death: Percentage of various forms	The day before death: No. of infected corpuscles counted	The day before death: Percentage of various forms	Heart's blood at the autopsy: No. of infected corpuscles counted	Heart's blood at the autopsy: Percentage of various forms	Total: No. of infected corpuscles counted	Total: Percentage of various forms	
I parasite	8,592	76·97 %	3,144	71·68 %	3,581	50·98 %	15,317	67·763 %	
II parasites	2,314	20·73	1,122	25·58	3,036	43·11	6,472	28·655	99·662 %
III ,,	57	·51	20	·46	83	1·17	160	·708	
IV ,,	191	1·71	91	2·07	291	4·12	573	2·536	
V ,,	3	·03	2	·04	4	·05	9	·039	
VI ,,	4	·03	5	·11	16	·22	25	·117	
VIII ,,	2	·02	2	·04	24	·34	28	·123	
IX ,,	—	—	—	—	1	·01	1	·004	·299 %
X ,,	—	—	—	—	1	·01	1	·004	
XII ,,	—	—	—	—	2	·02	2	·008	
XVI ,,	—	—	—	—	1	·01	1	·004	
	11,163	100·00	4,386	99·98	7,040	100·04	22,589	99·961	

From Table I (p. 251), in which the order has been arranged according to the degree of infection on the last day of life, it can be seen how greatly the degree of infection varies in acute cases.

Condition of infected cells. During the earlier stages of the disease the very great majority (70—90 %) of the infected corpuscles observed contained single round parasites (Plate IX, Fig. 1). Occasionally two rounded or pear-shaped forms were met with, but cells containing more than two parasites were very scarce. At a later stage of the disease corpuscles containing more than one parasite became relatively more numerous, and on the day of death only half the infected corpuscles contained a single parasite.

In Table II is given a summary of the observations made on films from all eleven dogs, recording the number of parasites present in the infected corpuscles two days before death and earlier, the day before death, and at the autopsy a few hours after death.

It is interesting to observe that of the 7,272 corpuscles containing more than one parasite (young forms) 97·66 % contain an even number of parasites and only 2·33 % an odd number.

Free parasites were seldom encountered in the earlier stages but subsequently became more numerous. Two and more days before death one free parasite to every 38 infected corpuscles was found. The day before death the proportion was one free parasite to 23 infected corpuscles, and on the day of death one free parasite to 18 infected corpuscles.

Phagocytosis. Instances of leucocytes containing infected, or non-infected, red corpuscles, were only observed frequently in three dogs. In Dog I numerous instances of phagocytosis were seen in films taken 7, 2 and 1 days before death. They were not observed between the 6th and 3rd day before death. In Dog II the phenomenon was noticed two days before death, but not subsequently, and in Dog XI seven days before death, but not later. In films from the other dogs examples were only rarely met with. In smears taken from the various organs examples of phagocytosis were uncommon.

Nucleated red cells. Nucleated red cells were common in films from Dogs I, II, IX taken the day before death, and in films from Dogs VIII, XI, and IV, taken 6th, 3rd, and 2nd days before death respectively, and during the intervening days.

Relation of degree of infection to the temperature. Parasites were occasionally met with in very small numbers just before the rise of temperature, but in the majority of instances they were first observed on the date on which a definite rise of temperature took place.

In the case of Dog XI (Chart II) the temperature rose on the 6th day from 101·2° F. to 103° F. and on the 7th day had reached 105·3° F. From the 7th to the 13th day it fell gradually to 100° F., and then again rose to 103·3° F. on the 16th day. On the 17th day it rapidly went down to 95° F., and the animal died. Parasites were first observed in very small numbers on the 6th day, and were numerous from the 7th to the 10th days. On the 12th, 13th, and 14th days, when the temperature was low, parasites were scarce, but gradually again increased in numbers as the temperature again rose.

In the case of Dog IX (Chart I) although the parasites first made their appearance when the temperature rose on the 12th day their numbers remained very small during the first rise and during the subsequent fall in the temperature, and only became numerous during the final period of high temperature.

Urine. Specimens of urine from 8 dogs were obtained from the bladder within a few hours of death. Except in Dog VIII the urine was thick, dark-coloured, resembling tincture of iodine, and gave a large, darkly coloured deposit on standing. It was acid in reaction. Six of the samples contained albumin in considerable quantities, and six contained bile pigment. Five showed casts and three blood corpuscles on microscopical examination of the deposit. Blood pigment was present in all examples of urine except that from Dog VIII, which was normal in every respect. The urine of two out of the three male dogs contained spermatozoa in large numbers.

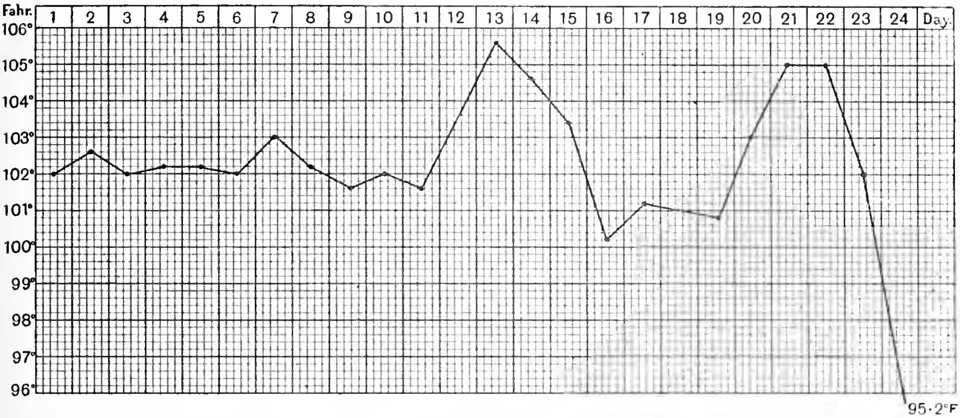
Appearances at the Autopsy.

Except that the mucous membranes were very pale and the subcutaneous tissues and fat more yellow than normal no constant gross lesions were observed at the autopsies. The stomach and intestines were usually empty, and the rectum loaded with bright yellow faeces. The urine in most cases was thick, and of a dark yellow colour, resembling tincture of iodine.

As shown in the table on pages 236–7 (Vol. IV, this *Journal*) other observers have laid stress on the enlargement of the spleen and con-

CHART I.

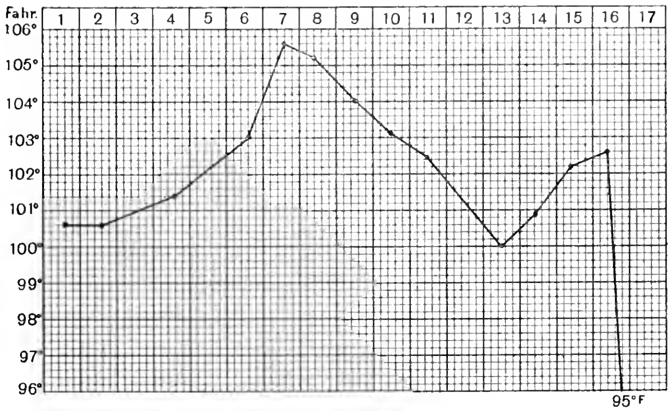
Dog IX. Infected by the subcutaneous injection of defibrinated blood from Dog VIII.



12th day, 4 parasites found. 13th day, 8 parasites found. 14th day, 34 parasites found. 15th day, 6 parasites found. 16th day, 11 parasites found. 17th day, 4 parasites found. 18th and 19th days, few parasites found. 20th day, parasites numerous. 21st, 22nd and 23rd days, parasites very numerous. 24th day, parasites very numerous (nucleated reds and phagocytes common).

CHART II.

Dog XI. Infected by the injection of 10 c.c. of defibrinated blood from Dog X.



6th day, 2 parasites found. 7th, 8th and 9th days, parasites numerous. 10th day, parasites numerous (many phagocytes). 11th day, parasites less numerous. 12th and 13th days, parasites few. 14th day, parasites few (nucleated reds common). 15th day, parasites more numerous (nucleated reds common). 16th and 17th days, parasites numerous (nucleated reds common).

gestion of the liver and spleen, with occasional enlargement of the former. Inflammation of the stomach and intestines has been especially frequently observed by Hutcheon.

In this series no macroscopic lesions were noted in Dogs IV, V, IX, X, and XI.

In Dog I the lungs were slightly oedematous. A few petechiae were observed in the stomach and omentum of Dog II. In Dog VI the lungs were oedematous, the spleen large and soft and the kidneys congested and very dark. In Dog VII the kidneys and suprarenals were congested and the spleen larger than normal. It contained three lymphomatous tumours. In the case of Dog VIII the spleen was large, soft and dark.

In some cases the blood was normal in appearance, but in Dog VII it was dark and of the consistency of treacle. In Dog III on the other hand the blood was represented by a yellowish fluid containing brown amorphous particles. This fluid became firmly clotted two hours after death.

The Histology of the Organs.

The organs were examined histologically by the following methods.

Small pieces were hardened either in Müller's reagent or in Bles' mercury solution, and cut in paraffin. Sections were stained by haematoxylin and counterstained by von Geison's method, and others were stained by Leishman's stain. In the latter case the sections, fixed on coverslips, were placed in distilled water containing a small quantity of Leishman's stain, and left for 12 to 24 hours. They were then placed in distilled water containing a drop or two of acetic acid for a few seconds, until the colour was pink, washed in distilled water, blotted dry, treated with xylol, and mounted in Canada balsam. By this means the parasites were brought out exceedingly well. In many cases other methods of staining were also adopted.

Smears on cover-glasses from the various organs were also prepared, stained by Leishman's stain in the same way as blood films and examined. From sections it was seen that in all cases the smaller capillaries contained more numerous infected corpuscles than the larger vessels. In the latter the infected corpuscles were generally to be found near the wall of the vessel. Consequently smears from the organs do not necessarily indicate the degree of infection, since the blood may be derived from any of the vessels. Care was taken to

obtain smears from places in which the larger vessels were not visible. Indications, however, may be obtained from the study of smears as to the degree of multiplication of the parasites within the blood corpuscles of the organ.

Lungs. The capillaries in the walls of the alveoli are as a rule dilated. Some of the alveoli are normal but in the majority there is proliferation of the lining cells, some of which are seen lying free in the air-cells. In other alveoli the process is more advanced, and numerous proliferated cells, leucocytes, and in some cases red-blood corpuscles are seen within them. The alveoli in which the process is more marked occur in groups of considerable size, which do not appear to have any relation to the bronchi or blood vessels.

Some of the bronchioles are normal, but many show proliferation of the epithelium, and the lumen frequently contains desquamated epithelium, leucocytes, and mucus.

There is no evidence of any increase in the connective tissue.

Blood vessels :—(1) The larger blood vessels appear to be normal but leucocytes are very common in them. Counts from the larger vessels showed between 12 % (Dog X) and 1 % (Dog VII) of infected corpuscles. (2) Smaller vessels show a larger number of infected corpuscles, which, as in the case of the larger vessels, generally lie close to the walls. (3) The capillaries in the alveolar walls are much dilated. Counts made from the capillaries in various sections showed between 52 % (Dog X) and 4 % (Dog VIII) of infected corpuscles.

The lungs of Dogs I, II, III, IX, and X showed the changes just described. In Dogs IV, V, VI and VII the changes were very marked. Enormous numbers of leucocytes were seen in the larger vessels, and there was much proliferation of the cells of the alveolar walls.

Smears show that 99·1 % of all infected corpuscles contain between one and four parasites, and only ·89 % contain more than four parasites. In the smears from Dog VIII one sausage-shaped organism (pp. 244, 245) was found, and in the same smears numerous forms with amoeboid processes were seen. Free parasites are very common, so that many can sometimes be counted in one field. The mean of all counts from lung smears shows that there are 1·5 free parasites to each infected red corpuscle.

TABLE III. *Summary of observations on all lung smears in regard to the numbers of parasites within infected corpuscles.*

No. of parasites within corpuscles	Infected corpuscles counted	Percentage of each variety	
I parasite	1,134	38·97 %	99·10 %
II parasites	1,524	52·37	
III ,,	31	1·06	
IV ,,	195	6·70	
V ,,	2	·07	·89 %
VI ,,	9	·31	
VII ,,	1	·03	
VIII ,,	9	·31	
X ,,	3	·10	
XIV ,,	2	·07	
	2,910	99·99	

Heart. The *muscle* is normal and its striation well marked. No pigmentary or fatty changes were observed. The *capillaries* lying between the muscular bundles are much dilated, and in some places slight haemorrhages have occurred. Infected corpuscles in the capillaries are very numerous. In Dog III 72 % of the red corpuscles in some of these vessels were infected. In Dog VI there was extreme dilatation of these vessels, but in Dogs VIII, IX, X and XI it was comparatively slight.

No changes were noted in the *skeletal muscles.*

Liver. The changes in this organ were more marked than in any other. The following are the lesions which occur in the majority of cases.

The central vein of the lobule and the capillaries between the liver cells are extremely dilated. The protoplasm of the liver cells stains badly, but the nuclei take the stain fairly well. The cells are distorted between the dilated blood vessels, and are in many cases almost destroyed, especially near the centre of the lobule. The vessels in the interlobular spaces seem dilated, but the bile ducts are normal. There is no increase of fibrous tissue, and the capsule is normal.

The capillaries contain large numbers of red blood corpuscles and the proportion of leucocytes is high. The latter are also very numerous in the larger vessels. In these vessels about 10 % of the red corpuscles are infected, and are usually to be found in small groups near the walls. The proportion of leucocytes to red corpuscles in them is about 1 to 10.

In the capillaries 23 % to 53 % (Dog VI) of the red corpuscles are infected, and in some instances the proportion of leucocytes to red blood corpuscles is as high as 1 to 3.

In Dogs VIII and IX the dilatation of the capillaries was not so marked as in the others, and although very numerous leucocytes were present in the vessels the number of infected red corpuscles was not very great. The liver was very fatty in Dog VIII. In Dog VII the fibrous tissue in the interlobular septa was much increased.

Smears show that 98·98 % of all infected corpuscles contain one to four parasites and 1·01 % contain more than four parasites. Free parasites are found in the proportion of one to 2·5 infected cells.

TABLE IV. *Summary of observations on all liver smears in regard to the numbers of parasites within infected corpuscles.*

No. of parasites within corpuscles	No. of infected corpuscles counted	Percentage of various forms	
I parasite	2,183	40·84 %	98·98 %
II parasites	2,677	50·08	
III ,,	67	1·25	
IV ,,	364	6·81	
V ,,	10	·19	1·01 %
VI ,,	12	·22	
VII ,,	3	·05	
VIII ,,	25	·47	
X ,,	2	·04	
XII ,,	1	·02	
XVI ,,	1	·02	
	5,345	99·99	

Spleen. The capsule and malpighian bodies are normal. The pulp contains in most cases a large quantity of blood, and the vessels in the trabeculae are dilated and contain numerous leucocytes.

The proportion of infected corpuscles in the pulp is small, varying between 3·7 % and 12 %, but in the smaller trabecular veins it is high, in one case up to 48 % (Dog I).

Smears show that 98·26 % of all infected corpuscles contain one to four parasites, and 1·73 % more than four. Free parasites occur in the proportion of 1 to 9·5 infected corpuscles.

TABLE V. *Summary of observations on all spleen smears in regard to the number of parasites within infected corpuscles.*

No. of parasites within corpuscles	Infected corpuscles counted	Percentage of each variety	
I parasite	2,474	46·27 %	98·26 %
II parasites	2,249	42·06	
III ,,	61	1·14	
IV ,,	470	8·79	
V ,,	14	·26	1·73 %
VI ,,	26	·48	
VII ,,	1	·02	
VIII ,,	45	·84	
X ,,	5	·09	
XIV ,,	1	·02	
XVI ,,	1	·02	
	5,347	99·99	

Kidneys. No changes were constantly observed in the kidneys except the dilatation of the blood vessels. The capillaries in the glomeruli are much distended, and so are the vasae rectae, and the vessels lying between the convoluted tubules. In most cases all the capillaries are crowded with infected corpuscles. For example in the section of a glomerulus, not differing from others (Dog I) 203 parasites were counted. In other cases about 46 % of all the red corpuscles seen in the vessels of the glomeruli were infected.

In the other capillaries of the organ large numbers of infected corpuscles were also counted, in some cases up to 95 % in the very small vessels. In Dog III about 50 % of all the corpuscles in these vessels were infected. The degree of infection, however, was not so great in Dogs IV and V, and in Dogs VIII and IX the dilatation was much less marked than in others, although the proportion of infected corpuscles was very high.

Smears show that although the proportion of infected corpuscles in the vessels of the kidney is very high the multiplication of the parasites within the corpuscles is not very great, for 99·01 % of all infected corpuscles contain one to four parasites, and only ·93 % more than four parasites. Free parasites occur in the proportion of 1 free parasite to 2·2 infected corpuscles.

Two of the sausage-shaped organisms described in the previous paper (p. 244) were met with in the kidney smears of Dog IX.

TABLE VI. *Summary of observations on all kidney smears in regard to the number of parasites within infected corpuscles.*

No. of parasites within corpuscles	Infected corpuscles counted	Percentage of each variety	
I parasite	2,802	48·37 %	99·01 %
II parasites	2,521	43·52	
III ,,	110	1·89	
IV ,,	303	5·23	
V ,,	10	·15	·93 %
VI ,,	23	·39	
VII ,,	2	·03	
VIII ,,	21	·36	
	5,792	99·94	

Suprarenal Capsules. The vessels of the suprarenal capsules both in the cortex and the medulla are dilated in the majority of cases. Otherwise the gland is normal. In Dog III about 70 % of the corpuscles in some of the smaller capillaries were infected.

Smears show that a comparatively large proportion of the infected corpuscles (1·91 %) contain more than four parasites. Free parasites occur in the proportion of one free parasite to 4·8 infected corpuscles.

TABLE VII. *Summary of observations on all suprarenal smears in regard to the number of parasites within infected corpuscles.*

No. of parasites within corpuscles	Infected corpuscles counted	Percentage of each variety	
I parasite	480	34·33 %	98·05 %
II parasites	727	52·00	
III ,,	24	1·71	
IV ,,	140	10·01	
V ,,	3	·21	1·91 %
VI ,,	11	·78	
VIII ,,	12	·85	
X ,,	1	·07	
	1,398	99·96	

Pancreas. In all cases the cells of the pancreas stain badly. The protoplasm is granular, especially at the inner zone, and the nuclei stain faintly, and in some cases can scarcely be made out. In all cases the capillaries and smaller vessels are greatly dilated and contain numerous infected corpuscles. The proportion of free parasites to infected corpuscles was one to nine.

TABLE VIII. *Summary of observations on all pancreas smears in regard to the number of parasites within infected corpuscles.*

No. of parasites within corpuscles	Infected corpuscles counted	Percentage of each variety	
I parasite	952	50·93 %	99·72 %
II parasites	825	44·14	
III ,,	10	·53	
IV ,,	77	4·12	
VI ,,	3	·16	·26 %
VIII ,,	2	·10	
	1,869	99·98	

Brain. Sections of various parts of the brain showed no changes except slight dilatation of the capillaries in the substance and meninges. In some cases there was an excess of cerebro-spinal fluid.

The degree of infection in the capillaries is in most cases high. In Dog III for example 42 red corpuscles were counted in one capillary of which 37 or 88 % were infected. Free parasites are very numerous in smears from the brain, the proportion being 1 free parasite to 2 infected corpuscles. The proportion of infected corpuscles containing more than four parasites is higher in the brain than in any other organ, namely, 4·91 % of all infected corpuscles.

TABLE IX. *Summary of observations on all brain smears in regard to the number of parasites found within infected corpuscles.*

No. of parasites within corpuscles	Infected corpuscles counted	Percentage of each variety	
I parasite	304	38·33 %	95·07 %
II parasites	317	39·97	
III ,,	16	2·02	
IV ,,	117	14·75	
V ,,	2	·25	4·91 %
VI ,,	11	1·38	
VIII ,,	20	2·52	
XII ,,	2	·25	
XIV ,,	1	·13	
XV ,,	1	·13	
XVI ,,	2	·25	
	793	99·98	

Spinal Cord. Nothing abnormal was noticed except slight dilatation of the vessels of the substance and meninges.

Small Intestine. The vessels of the villi are much dilated and

crowded with infected corpuscles. A considerable number of leucocytes are found in the vessels and connective tissue spaces.

Mesentery and Omentum. Pieces of the mesentery and omentum were stretched out on cork, fixed by formalin vapour, and stained by Leishman's method. In nearly all the specimens the smaller vessels were seen to be crowded with infected corpuscles and leucocytes (Plate X, Fig. 2).

Lymphatic glands. Except for the dilatation of the smaller vessels the lymphatic glands are normal. Free parasites occur in the smears in the proportion of one free parasite to 2·2 infected corpuscles. The number of infected corpuscles containing more than four parasites is higher than any other organ except the brain (4·74 %).

TABLE X. *Summary of observations on all lymphatic gland smears in regard to the number of parasites found within infected corpuscles.*

No. of parasites within corpuscles	Infected corpuscles counted	Percentage of each variety	
I parasite	253	40·03 %	95·25 %
II parasites	291	46·04	
III ,,	2	·32	
IV ,,	56	8·86	
V ,,	1	·16	4·74 %
VI ,,	6	·94	
VII ,,	1	·16	
VIII ,,	17	2·69	
X ,,	2	·31	
XII ,,	1	·16	
XVI ,,	2	·32	
	632	99·99	

Marrow. Smears were made from marrow taken from the shaft of the femur.

As will be seen from the following table the proportion of corpuscles containing two and more parasites was very high. Corpuscles containing 4, 6, and 8 parasites amounted to 15·38 % of all of those infected, and corpuscles containing more than 4 parasites to 3·94 %. The examination of these films in which there were large numbers of nucleated red cells only showed one infected with parasites (Plate IX, Fig. 45). The percentage of infected corpuscles was very high, but varied greatly in the different dogs. Free parasites occurred in the proportion of one free parasite to 8 infected red corpuscles.

Summary.

In the present series of acute cases no constant macroscopical lesions were found at the autopsies. Histologically it was found that the blood vessels of the organs were dilated, and contained very large numbers of leucocytes. In the majority of cases parasites were seen in great numbers in the small capillaries, a high percentage of the blood corpuscles being infected. In the larger vessels a much smaller proportion of the corpuscles contained parasites, and these were usually found near the periphery of the vessel.

Table XI. *Summary of observations on all marrow smears in regard to the number of parasites within infected corpuscles, and* Table XII, *showing percentage of infected red corpuscles.*

Table XI.

No. of parasites within corpuscles	Infected corpuscles counted	Percentage of each variety	
I parasite	2,562	29·59 %	
II parasites	4,508	52·06	95·98 %
III ,,	168	1·94	
IV ,,	1,083	12·39	
V ,,	35	·40	
VI ,,	103	1·19	
VII ,,	11	·18	
VIII ,,	156	1·80	
IX ,,	3	·03	
X ,,	10	·11	
XII ,,	2	·02	3·94 %
XIII ,,	1	·01	
XIV ,,	1	·01	
XVI ,,	13	·15	
XVII ,,	2	·02	
XVIII ,,	1	·01	
XXI ,,	1	·01	
	8,660	99·92	

Table XII.

Dog	Percentage of infected corpuscles
VI	53·5 %
XI	42·5
X	37·0
II	35·0
IX	22·0
III	21·0
VII	11·3
I	9·0
V	4·5
IV	2·0
VIII	2·0
Mean	22 %

Corpuscles containing more than four parasites were found in the greatest numbers in the brain, lymphatic glands, and marrow. It is therefore probable that multiplication within the corpuscles is more rapid in the capillaries of these organs than in other situations.

TABLE XIII. *Showing the relative proportion of infected corpuscles containing more than four parasites.*

Brain	4·91 %	Kidney	·93 %
Lymphatic glands	4·74	Lung	·89
Marrow	3·94	Heart blood...	·66
Suprarenal capsules ...	1·91	Pancreas	·26
Spleen	1·73	Blood (day before death) ...	·19
Liver	1·01	,, (2 and more days before death)	·08

The liver showed changes due principally to the pressure of the dilated capillaries, and the lungs were in most cases in a condition resembling bronchopneumonia.

Phagocytosis of the infected cells was common in some cases a few days before death, and nucleated red cells were frequently encountered in the peripheral circulation towards the end of the disease.

TABLE XIV. *Showing the relative frequency of the occurrence of infected red corpuscles containing various numbers of parasites compiled from all the observations made on organ smears and blood films.*

No. of parasites in infected corpuscles	No. of infected corpuscles counted	Percentage of each variety	
I parasite	28,414	51·196 %	98·672 %
II parasites	22,286	40·155	
III ,,	666	1·200	
IV ,,	3,397	6·121	
V ,,	89	·160	1·326 %
VI ,,	231	·416	
VII ,,	19	·034	
VIII ,,	334	·602	
IX ,,	4	·007	
X ,,	24	·043	
XII ,,	8	·014	
XIII ,,	1	·001	
XIV ,,	4	·007	
XV ,,	1	·001	
XVI ,,	20	·036	
XVII ,,	2	·003	
XVIII ,,	1	·001	
XXI ,,	1	·001	
	55,502	99·998	

The preceding table, compiled from all the observations on films from the peripheral blood and smears from organs, shows the relative frequency of blood corpuscles containing various numbers of parasites.

It shows that over 91 % of all infected corpuscles contain either one or two parasites only, and that 98·6 % contain one to four parasites.

Even numbers of parasites are present in 97·11 % of all infected corpuscles containing more than one parasite and odd numbers in only 2·88 %.

Free parasites are found most frequently in lung smears, next most frequently in brain, lymphatic gland and liver smears, and less frequently in smears from other organs. At all times they are less common in the peripheral circulation.

The following table gives the proportion of free parasites to infected corpuscles in smears from the different organs and blood films.

TABLE XV. *Showing the proportion of free parasites to infected corpuscles.*

Organs					
Lungs	1·5 free parasites	to	1	infected	cell.
Brain	1	,,	2	,,	,,
Lymphatic gland	1	,,	2·2	,,	,,
Kidney	1	,,	2·2	,,	,,
Liver	1	,,	2·5	,,	,,
Suprarenal gland	1	,,	4·8	,,	,,
Marrow	1	,,	8	,,	,,
Pancreas	1	,,	9	,,	,,
Spleen	1	,,	9·5	,,	,,
Blood (autopsy)	1	,,	18	,,	,,
,, (day before death)	1	,,	23	,,	,,
,, (2 and more days before death) ...	1	,,	38	,,	,,

The urine taken from the bladder at the autopsy is generally very darkly coloured, and contains albumen, bile salts, and blood pigment, and in the case of male dogs spermatozoa.

EXPLANATION OF PLATES X AND XI.

All specimens stained by Leishman's stain by the method described on p. 256.

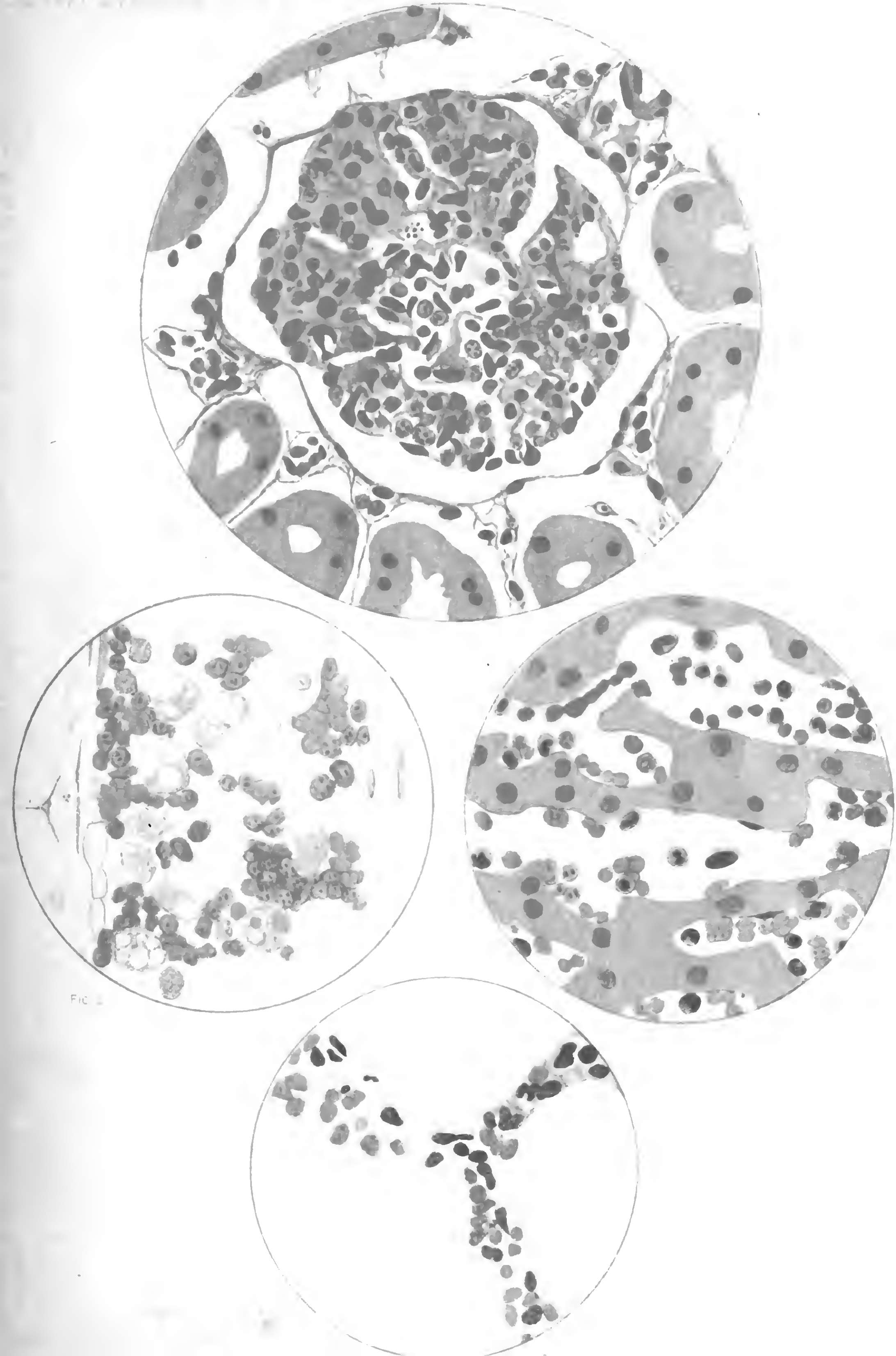
PLATE X.

Fig. 1. Glomerulus from the kidney of Dog I, showing very numerous infected red corpuscles in the capillaries.

Fig. 2. Portion of a small vessel from the omentum of Dog V, showing large numbers of infected red corpuscles and numerous leucocytes.

Fig. 3. Part of the liver of Dog I, showing greatly dilated capillaries containing many infected red corpuscles and numerous leucocytes.

Fig. 4. Portions of the walls of lung alveoli showing numerous infected corpuscles in the capillaries.



FIG

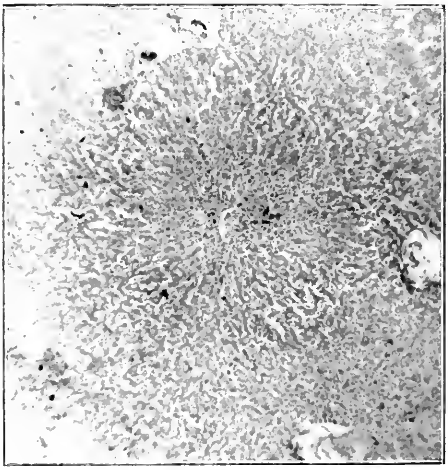


Fig. 1.

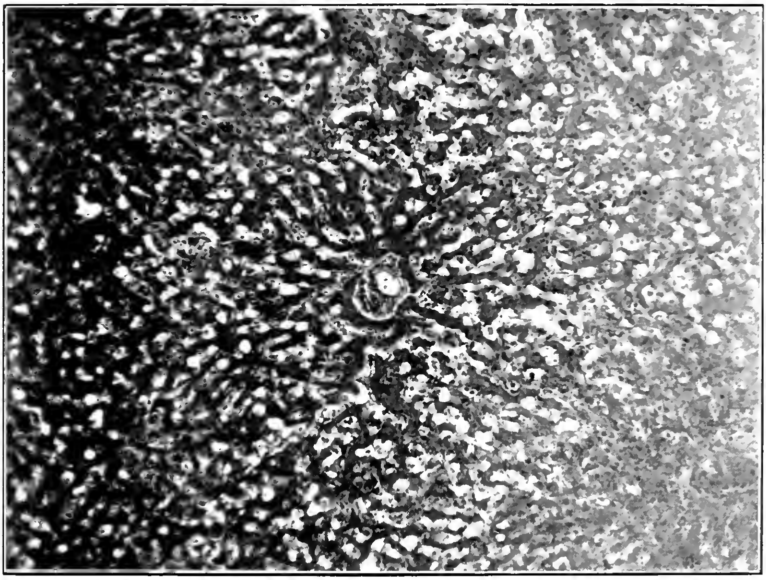


Fig. 2.

PLATE XI.

(1) Micro-photograph of a lobule of the liver showing at the periphery dilated capillaries (white) between the liver cells (dark). The capillaries can be traced almost up to the central vein. In this region the liver cells are much atrophied and numerous leucocytes are present in the capillaries. (Magnification about 45.)

(2) Micro-photograph of the periphery of a lobule showing the greatly dilated capillaries (white) containing numerous leucocytes (dark nuclei). Liver cells dark. (Magnification about 66.)

CANINE PIROPLASMOSIS. IV.

ON CERTAIN CHANGES IN THE BLOOD.

By J. ALDREN WRIGHT, M.D.

(From the Pathological Laboratory, Cambridge.)

Having examined the blood in a number of the dogs suffering from Piroplasmosis, referred to in the previous papers, I have selected three charts to show the changes I found to take place in the blood during the progress of the disease. For the enumeration of the corpuscles the Thoma-Zeiss haemacytometer was used, and for estimating the haemoglobin, Haldane's modification of Gower's haemoglobinometer.

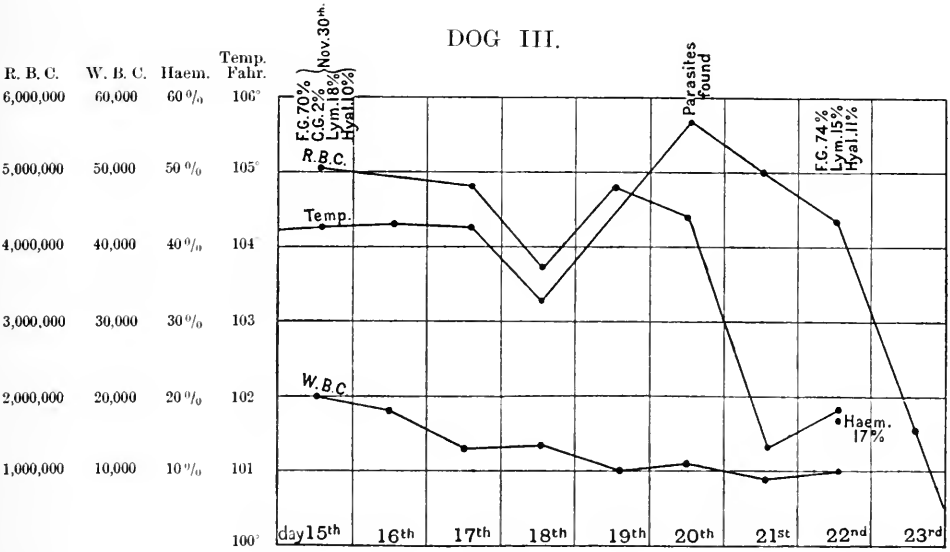
Taking first the number of the red corpuscles, a great reduction was a constant feature. In one dog the number fell from 5,800,000 per c.mm. to 1,000,000, and in another from 5,000,000 to 950,000, on the day previous to the animal's death. The red corpuscles were also altered in character, some were smaller than normal, while others were considerably larger, and these latter stained faintly. When the parasites appeared in the blood nucleated red cells were also to be found but usually few in number. In one instance (Dog XI, July 8th) enormous numbers were present, almost equalling in number the polynuclear leucocytes. A comparative count gave 48 °/₀ nucleated red cells to 52 °/₀ polynuclear leucocytes. As many as eight nucleated red cells could be seen in a single field of a $\frac{1}{12}$ inch objective. In this case the red corpuscles were altered in size and shape and many of them were broken up.

The haemoglobin was always diminished in amount, closely following the diminution in the number of red corpuscles. The smallest amount found was in Dog XI, in which it was only 17 °/₀ of the normal.

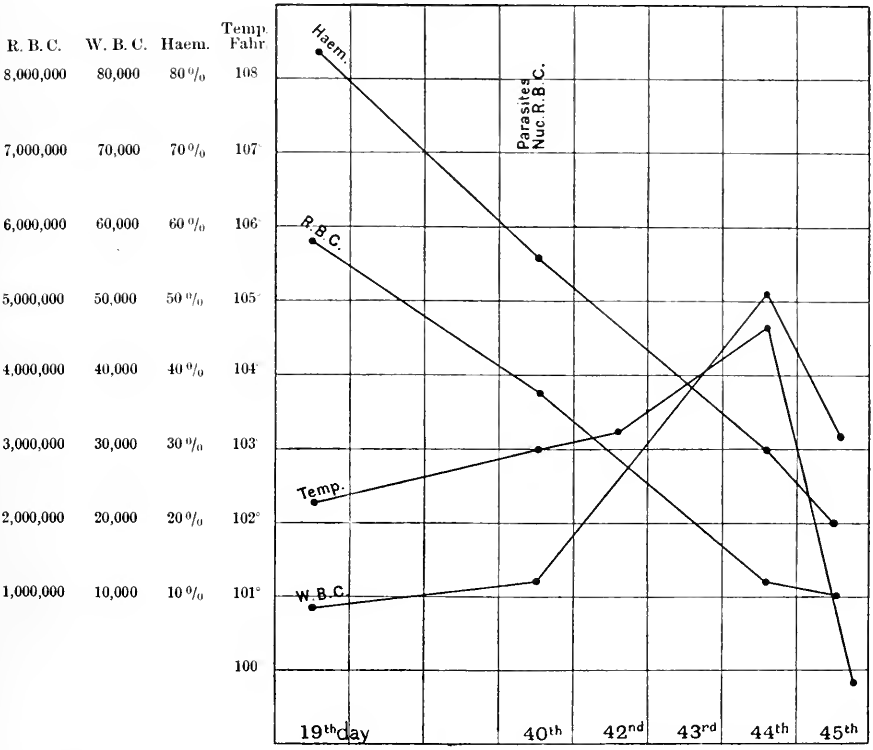
Usually the leucocytes were greatly increased from the time the parasites appeared in the blood. In one dog they numbered nearly 52,000 per c.mm. and in another they increased to 60,000.

On the contrary in Dog III there was a diminution in the number of the leucocytes. Both the polynuclear and the mononuclear forms appeared to share in the general increase.

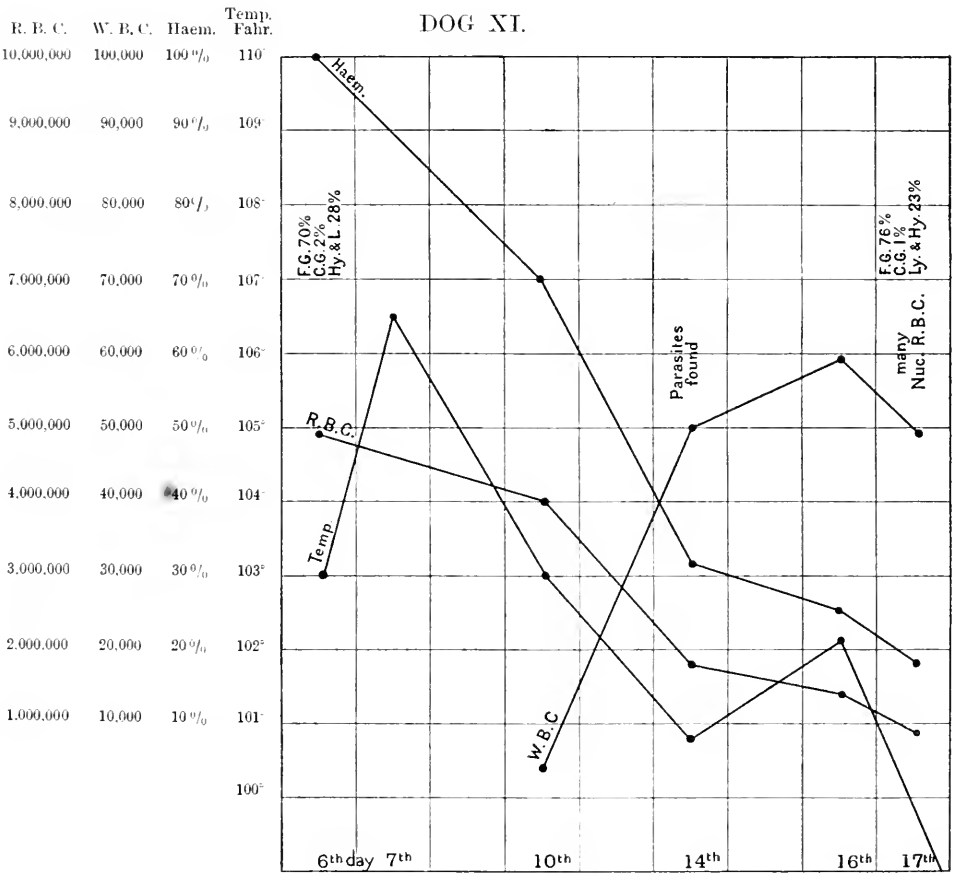
DOG III.



DOG VIII.



DOG XI.



Abbreviations in the Charts.

Haem. = Haemoglobin.
Temp. = Temperature.
F.G. = Finely granular Leucocytes.
C.G. = Coarsely „ „

R.B.C. = Red blood corpuscles.
Nuc. R.B.C. = Nucleated red blood cells.
Hy. & Ly. = Hyalines and Lymphocytes.
W.B.C. = Leucocytes.

A NOTE ON BOVINE PIROPLASMOSIS.

By A. E. METTAM, B.Sc., M.R.C.V.S.,

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So much has been written concerning *Piroplasmosis* as it occurs in different animals in various parts of the world that one may be pardoned for directing attention to the fact that the disease exists at home and is constantly coming before the notice of the veterinarian at this time of the year.

The affection receives a variety of names according to the district, but that of redwater is common enough, others, as red or blood murrain or moor ill or evil, may not be so general. Many causes were assigned as the primary agent in the etiology of redwater, but we now know that the redwater seen in bovines of both sexes when at grass is due to the presence in the red corpuscles of the protozoon, the *Piroplasma bigeminum.* There is a form of redwater prevalent at certain times of the year among parturient bovines but the cause of this so far as I know is not understood. It is probably due to some other cause than the *Piroplasma.* I have had no experience, however, of the disease and can give no opinion.

For several years past, since 1901, I have been aware of and have taught the presence of the *Piroplasma* in the blood of bovine animals suffering from haemoglobinuria[1], and through the courtesy of many members of the profession in Ireland I have received specimens of blood from many different parts of the country. Rarely have I failed to demonstrate the parasite. Quite recently I have had experience of three different outbreaks and in all the blood showed the Piroplasmata in abundance.

The tick found upon the affected animals is the *Ixodes reduvius*[2].

[1] There is a haemoglobinuria affecting equines in the winter months particularly, known commonly in the veterinary profession as azoturia, but the cause is unknown.

[2] Specimens of these ticks were sent to Dr Nuttall for identification.

It is generally admitted that young bovines possess a considerable amount of immunity to the disease, but I have reason to believe that this is not so great, at any rate in animals approaching a year old, as is supposed. Young bovines, yearlings, do suffer from redwater and badly. Quite recently I saw two young bullocks, the urine in each case was the colour of port wine, the temperature 105° F., with numerous Piroplasmata in the blood. Both animals made good and speedy recoveries in contradistinction to a year-old heifer and an aged cow. The latter died, the heifer had a prolonged convalescence. On the same farm where these cases occurred two young bullocks had died prior to my visit from the disease. More than 50 cases have occurred on this farm this year.

In another case observed more recently the Piroplasmata were readily found in the blood of a milch cow. She is recovering, and her milk which had ceased at the onset of the attack is returning and I understand her urine is quite normal in appearance. Parasites though not numerous can be found without difficulty, 2–4 infected corpuscles being found in a field of the immersion lens. Apparently blood corpuscle destruction has ceased, but a very interesting fact was noted at the time of the appearance of the high-coloured urine and before the milk had fallen away, and that is a discoloration of the milk, evidently a tinging of the milk plasma with the haemoglobin in solution.

It is well known to those who occupy farms where animals are subject to redwater that the cattle reared upon the farm enjoy an immunity to the disease which attacks and rapidly kills off animals purchased and brought on to the farm. The explanation is probably that the natural immunity possessed by the young bovine is rendered active and durable by an attack of *Piroplasmosis*, which passes unperceived; that during the immunity period infection occurs which is thrown off, but it is sufficient to render the animal immune to redwater during the remainder or greater part of its life. I am convinced that young bovines do suffer from *Piroplasmosis* and that the disease becomes chronic, and that it claims many victims.

I have recently had under observation a number of young animals suffering from a disease of a very obscure nature. The symptoms were those of chronic persistent anaemia, great wasting, and colliquative diarrhoea. The temperature remained normal, but the appetite was fickle and often absent. On post-mortem examination, save for the liver and gall-bladder, there was nothing apparent to account for the condition. The liver was, in most cases, not in all, infected with fluke (*Distoma hepaticum*). The liver was cirrhotic and enlarged, the gall-bladder

dilated enormously and filled to repletion with bile. In one case 80 fluid ounces of bile were measured ($2\frac{2}{7}$ litres). The results of bacteriological examination have been negative. Prolonged examination of the blood carried over several months has demonstrated isolated *Piroplasmata* in the resting condition in the last schizogonous stage. Exceptionally a twin parasite has been found in experimentally infected animals. Massive inoculations of blood into healthy young cattle of approximately the same age have given reactions of temperature of approximately 5° F., after an incubation period of seven days, and the animals are steadily losing condition. The *Piroplasmata*, few in number, are to be found in the blood corpuscles. In this form of the disease there is no haemoglobinuria. We intend continuing the work and instituting some tick experiments and hope later to publish our results.

A note on the Sarcocystis tenella.

Sarcosporidiosis is not uncommon in the heart muscle of sheep where the encysted parasite may be observed lying dormant as a deeply stained, sharply delimited body in the muscle fibre. Quite recently my colleague (Prof. G. H. Wooldridge) and myself have found the spores in relatively large numbers in sheep dying from an affection believed at first to be ovine *Piroplasmosis*. The spores are banana-shaped with one extremity pointed, they average 14μ in length by 5μ in width. Stained by the Romanowsky method the pointed extremity stains with eosin, the body of the spore which is granular stains blue. The nucleus, which is clearly differentiated, possesses a well-defined nuclear membrane, is placed towards the blunt end, and has the chromatin stained purplish-red. I have seen the "spore" in the blood. Like Laveran and Mesnil we have failed to find any filament projecting from the anterior extremity. As a minor point I have seen the encysted parasite in Purkinje's fibres of the heart muscle, a situation in which Doflein (1901, *Die Protozoen*, p. 221) states they have been found and figured by Schneidemühl. Since this note was written I have observed numerous spores in a preparation made from the heart of a young bovine.

THE PREVALENCE OF TRICHOCEPHALUS DISPAR.

By H. S. FRENCH, M.D., M.R.C.P.,
and A. E. BOYCOTT, M.D.

(From the Gordon Laboratory, Guy's Hospital.)

No investigations seem to have been made in recent years as to the prevalence of intestinal worms among the general population in this country. The present results are derived from the microscopical examination of the stools of 500 in-patients of Guy's Hospital. The cases were taken consecutively by beds all through the hospital, without any selection, over a period of ten months, and comprise surgical and medical as well as gynaecological and other special patients. Nearly all were inhabitants of London.

The method of examination was to shake up a small portion of the faeces with normal saline solution in a test-tube; after allowing the mixture to sediment for about half-an-hour the supernatant fluid is poured off and the deposit shaken up with fresh saline. This process is repeated four or five times, and by it all the finer *débris* is removed; one drop of the final deposit containing the eggs in a concentrated form is mounted fresh, and any eggs present are readily detected under a low power. The method is chiefly of value where very few eggs are present. In such cases eggs are often found where a negative result has been obtained in the direct examination of the unwashed faeces. Unless the time allowed for sedimentation is unreasonably curtailed, there is no danger of eggs being lost during the washing. In more than four-fifths of the cases each sample was examined independently by two observers, the whole of the deposit under a $\frac{7}{8}$ in. square cover-glass being searched methodically on a mechanical stage.

Eggs of parasites were found in 40 cases; in one *Ascaris* eggs were discovered with difficulty in a boy aet. 6, who had been admitted to the

hospital as a case of *Ascaris* infection; in the remaining 39 eggs of *Trichocephalus dispar* were found. We did not meet with the eggs of any other parasite. The following table shows the details of these cases with regard to age distribution:

Age	Total cases examined	Cases in which eggs were found	Percentage infected
0—5	42	1	2·4
5—10	43	5 (+1 *Ascaris*)	11·9
10—20	86	9	10·5
20—30	96	12	12·5
30—40	58	7	12·1
40—50	69	3	4·4
50—60	51	2	4·0
60—70	19	0	0
over 70	3	0	0
age not given	33	0	0
	500	39 +1 *Ascaris*	7·8

These figures indicate a well-marked susceptible period of life; 84 p.c. of the infections fall between 5 and 40, while but 57 p.c. of the cases examined come within that age period. The absence of infections in children is striking; the single case in the period 0–5 years was aged 4, so that in the first four years of life we have 41 cases with no infections[1].

The sex incidence is equal: of 279 males 22 (= 7·9 p.c.), and of 221 females 17 (= 7·7 p.c.), were found to be infected.

As might be expected, nothing suggestive is found in the diseases with which *Trichocephalus* was associated. Roughly speaking 45 p.c. of the infected patients were medical, 30 p.c. surgical, 7 p.c. gynaecological, and 10 p.c. were suffering from affections of the eyes. Of 24 cases of appendicitis, two (8·3 p.c.) had worms; as this is rather less than the average incidence (9·4 p.c.) in all the cases of the same age period, no support is afforded to the notion that Trichocephalus has any aetiological relationship to appendicitis[2]. The length of time between the patients' admission to the hospital and the discovery of eggs varied from 2 to 103 (average 27) days. In no case were the eggs numerous, and in the majority not more than 1 to 3 were found in a drop of the washed faeces.

[1] The presence of *Oxyuris* is often missed by the ordinary methods of examination of faeces. We have had the opportunity of comparing in a number of cases the results of the microscopical examination of faeces with a subsequent microscopical search for the adult worms after the administration of thymol. In the former the eggs or worms were seen extremely seldom, while in the latter specimens of *Oxyuris* were nearly always found.

[2] Metchnikoff, *Bull. de l'Acad. de Méd.* vol. XLV. 1901, p. 301.

The results have indeed no pathological interest; they indicate rather the extent to which the general population in London comes into contact with human faeces. The life-history of *Trichocephalus dispar* is not elucidated in all detail, but the essential facts seem to be fully established. The eggs measure about 55 by 25 μ, and as they leave the host in the stools contain an undifferentiated ovum; further development is very slow and many months are occupied in the growth of the larva within the egg. This growth is to some extent independent of temperature and will take place at "room-temperature" in this country. The thick egg-shell is very resistant; in consequence the embryo can survive great vicissitudes of temperature and moisture, and may remain capable of development for a long time (up to five years—Davaine) after leaving the body. The larva does not escape from the egg outside the body, but only on being taken into the alimentary canal. Eggs which do not contain a developed larva are not infective. As far as is known, infection can only take place *per os*. The length of life of the adult worm in the human intestine is, we believe, quite unknown; analogy would lead us to suppose that it may extend to several years.

Monkeys and lemurs are said to be often infected with *T. dispar*; in this country, however, this is of no moment in the present connection. Closely allied, but distinct, species are found in several of the domestic animals—notably *T. crenatus* in the pig and *T. affinis* in the sheep. The eggs of these other species very closely resemble those of *T. dispar*, but the specificity of worm infections practically precludes the suggestion that the eggs which we have found in human stools are other than those of *T. dispar*, and that the infections with this worm had their origin in faeces other than human[1].

It follows from this that infection arises by the ingestion of material contaminated with stale human faeces. Immediate personal infection or reinfection is excluded by the fact that the eggs must go through a lengthy period of development outside the body before they become infective. Any interest which our result may have lies in the demonstration—which is we believe unequivocal—that all the precautions which are taken in this country to secure the safe disposal of human excreta have not been adequate to prevent a purely faecal infection being present in at least 8 p.c. of the population[2].

[1] It is possible, though hardly credible, that the eggs found might have been the eggs of some other species of *Trichocephalus* which had been swallowed and passed through the body as such. In any case, the infection must be faecal whether it be human or animal.

[2] It follows from the long duration of the infection that this figure is to some extent an accumulated result, and possibly also partly the result of conditions now past.

On the other hand it is satisfactory to note that these results indicate a far more efficient cleanliness than do similar statistics from other places, a selection of which follows:

Place	Number examined	Percentage with *Trichocephalus*
Erlangen[1]	1755	11·1
,, (insane)[1]	138	100
Dresden[1]	1939	2·5
Kiel[1]	1117	32·2
Dublin[2]	90	90
Greenwich[2]	16	69
Paris[3]	?	50
Naples[3]	80	100
Bâle[4]	752	23·6
United States (insane)[5]	500	10·8
Porto Rico[6]	5490 (?)	6 +
Cornish Miners[7]	48	79
India[8]	1249	4·4
,, [9]	?	90

The cause and nature of the infection are such that, apart from variations in the method and thoroughness of the examination, the degree of infection must vary quantitatively with the degree of faecal contamination. This can be estimated directly only by an intimate knowledge of the details of the habits of the people concerned. The only instance which has come under our personal observation fully supports the connection: Cornish miners have hitherto worked in very filthy surroundings and are infested with worms. Many of the statistics quoted refer to the experience of some thirty years ago, and it may well be that much improvement has taken place since then.

As far as our own cases are concerned, the paths of infection cannot be definitely traced; the absence of the worm in young children[10]

[1] Leuckart, *Transl. Hoyle*, I. 1887, p. 151. [2] Cobbold, *Parasites*, 1879, p. 179.

[3] C. Davaine, *Traité des Entozoaires*, 1877, p. 209.

[4] Blanchard, *Traité de Zoologie Médicale*, 1889, I. p. 783.

[5] Bull. no. 13, Hyg. Lab., U.S. Pub. Health and Mar.-Hosp. Serv., Washington, 1903; other statistics will be found here.

[6] Report of Commission on "Anemia" in Porto Rico, San Juan, 1904.

[7] This *Journal*, IV. 1904, p. 477; subsequent experience has fully confirmed this figure.

[8] Dobson, *Report on Ankylostomiasis*, 1892.

[9] Hektoen and Riesman, *Pathology*, I. 1901, p. 344.

[10] This point is brought out in the Erlangen statistics (children 4·8, adults 13·1 p.c.), but not in those from Kiel (children 32·5, adults 29·5 p.c.). Blanchard, *Traité de Zool. Méd.* I.

suggests somewhat strongly that infection is brought about by the injection of "ordinary" food, since in other ways children would appear

Sex and age	Disease	Differential leucocyte count: Percentages Lympho-cytes	Inter-mediate	Large hyaline	Neutro-phile	Eosino-phile	Mast-cells[1]
M 36	aneurysm of aorta	9	15·5	19	55·5	**1**	0
M 39	chronic nephritis	27	21	6	42·5	**2·5**	1
M 31	crushed hand	21	19·5	3·5	53·5	**1**	1·5
M 12	mastoid disease	14	9	6·5	68·5	**2**	0
M 24	hip disease	14·5	11	6·5	63·5	**4**	0·5
M 25	acute rheumatism	21·2	20·2	3·2	51·6	**3·8**	0
M 25	detached retina	23·5	15·5	14·5	45·5	**1**	0
M 23	acute pericarditis	23·4	10·4	1·2	61·4	**3·2**	0·4
M 48	hemiplegia	28	11	2	56·5	**1·5**	1
M 28	mitral disease	31	12	3	53·5	**0·5**	0
M 16	sciatica	36·5	11	1·5	47	**3·5**	0·5
M 52	hemiplegia	23	17	2	56	**2**	0
M 7	empyema	12·5	5	1·5	80·5	**0·5**	0
M 20	detached retina	28	15	4·5	46·5	**4·5**	1·5
M 24	sight failure	25	16·5	8·5	49	**1**	0
(M 6	*Ascaris*	34	7·5	6	49	**3 5**	0)
F 38	phthisis	22	8	2	66·5	**0·5**	1
F 19	endometritis	20	6	2	70	**1·5**	0·5
F 40	?	5·5	6·5	1·5	86	**0·5**	0
F 23	chlorosis	20	13	4	57	**5·5**	0·5
F 8	pneumonia	32	9·5	3·5	53	**2**	0
F 19	acute rheumatism	31·5	6	2	59	**1·5**	0
F 35	lupus	36	6	4·5	49	**3·5**	1
F 9	hip disease	27·5	8·5	2·5	57·5	**3**	1
F 11	acute rheumatism	46	7·5	4	41·5	**0·5**	0·5
F 23	gastric ulcer	23·5	6	2·5	62·5	**3·5**	2
F 16	exophth. goitre	40	6·5	2	50	**1·5**	0
					Av.	**2·1**	

to be particularly liable to take in undesirable material. Among the common articles of diet, the readiest mode of infection is offered by uncooked vegetables, and perhaps especially by water-cress, which is eaten very commonly by the poorer classes in London, and which is often derived from sources which are open to the gravest suspicion of sewage contamination. The greater part, at any rate, of the London water is subjected to some sedimentation before delivery; as the eggs settle very quickly, this would seem to exonerate the water supply, apart from the influence of filtration[2].

[1] Mast-cells were always present, though none were found in cells actually enumerated.
[2] Cf. Blanchard, *Archives de Parasitologie*, III. 1900, p. 485.

The opportunity has been taken of investigating the condition of the eosinophile leucocytes in some of the cases. The table on the preceding page of 26 cases confirms the opinion previously expressed[1] that *Trichocephalus* infection, at any rate of the mild degree which was here present, is not accompanied by an eosinophilia.

We have much pleasure in acknowledging the invaluable help which has been rendered by our laboratory assistant, J. R. Clark, in obtaining these results, which, without such efficient aid, would have been long delayed.

[1] This *Journal*, IV. 1904, p. 468.

A CASE OF SKIN INFECTION WITH ANKYLOSTOMA.

By A. E. BOYCOTT, M.A., M.D.

(From the Lister Institute of Preventive Medicine.)

In view of a previous failure[1] to produce a skin infection with *Ankylostoma*, it seems worth while to put on record the present successful case. Dr J. B. Leathes very kindly offered himself as the subject of experiment. It is important to note that he has never, either before or during the course of the experiment, had anything to do with any *Ankylostoma* material except that deliberately applied to his person for the purpose of this experiment, nor has he been to any infected place or even worked in the part of the Institute where the cultures of *Ankylostoma* larvae have been kept. On March 21st a small quantity of water containing encapsuled larvae of *A. duodenale*, hatched artificially from infected faeces from Cornwall, and 13 days old, was applied to the forearm. After five minutes the arm was bandaged up and left for two hours, at the end of which time the wrappings were removed and the arm thoroughly cleansed. The purpose of the experiment and the precautions necessary were fully appreciated by the subject, and there is no doubt that there was not the slightest possibility of a mouth infection.

A definite eosinophilia was found on April 17 (27 days). Eggs were looked for on May 4 and May 6 without success, but on May 10 (50 days) a few eggs were found. Thymol (40 grains) was given on May 13 (53 days), but on May 22 one or two eggs were still present. On May 27 (67 days), 90 grains of thymol were taken in three 30-gram doses, and on June 6 a single egg was found with some difficulty. This failure to effect complete cure immediately after infection is possibly due to the varying times taken by different larvae to reach the intestine.

[1] This *Journal*, vol. IV. 1904, p. 89.

The symptoms observed were slight, though definite. The morning after the application of the larvae half-a-dozen small roundish patches of erythema were present in the treated area; these were not raised and faded completely on pressure. They made no further progress and in a few days had completely disappeared. Itching was felt for the first time a few hours after the larvae had been washed off the arm and continued for about a fortnight. Symptoms of "dyspepsia," hardly amounting to more than epigastric discomfort, were noticed after about fourteen days, and lasted till thymol was taken; after this they diminished, but were still present to some extent. The subject of the experiment is normally altogether a stranger to such symptoms, which seem to be just the same as those previously experienced by the writer after infection[1], to which they are doubtless due. Somewhat indefinite symptoms of bronchial catarrh were present from the second week onwards. No anaemia was produced.

The blood, and more especially the differential leucocyte count, was examined from time to time. The chief results are given in the following table:

Days after infection	Haemoglobin per cent.	Leucocytes per cub. mm.	Lymphocytes	Intermediate	Large hyaline	Neutrophile	Eosinophile	Mast-cells
0			20·0	12·4	4·6	61·0	**2·0**	0[2]
14			9·8	14·0	5·2	62·2	**7·4**	1·4
21			29·0	5·0	3·4	58·2	**3·0**	1·4
27	102	8000	8·4	17·6	6·0	53·2	**14·4**	0·4
43			13·2	7·8	5·4	60·0	**13·4**	0·2
44	105	7000	19·6	8·0	3·6	48·0	**20·8**	0
48			12·8	9·6	2·4	38·8	**35·6**	0·8
50		Eggs found						
51			15·6	3·4	0·6	52·0	**28·0**	0·4
52	98	10600	10·8	5·2	3·2	35·2	**44·8**	0·8
53		Thymol grains 40						
55	102	8100	19·6	5·4	2·0	40·0	**31·0**	2·0
64	96	8800	19·2	2·8	2·0	54·4	**21·6**	0
67		Thymol grains 90						
69	100	7800	24·4	5·6	2·0	46·0	**20·8**	1·2
72		7100	13·6	11·6	1·6	56·0	**17·2**	0
95			21·6	4·0	3·2	47·2	**22·8**	1·2

It will be seen from this that a definite eosinophilia was found 27 days after infection and 23 days before eggs appeared in the stool.

[1] This *Journal*, vol. IV. 1904, p. 463; see also C. A. Smith, *Journ. American Med. Association*, vol. XLIII. 1904, p. 592.

[2] Mast-cells were constantly present, though not always found in the leucocytes enumerated. The counts were all made at about the same time of day, fasting.

The eosinophilia subsequently rose to a very high figure (45 p.c.), subsiding somewhat after treatment. The total leucocytes were once observed to exceed 10,000, but even at the height of the eosinophilia there was no marked leucocytosis. Such increase as there was is fully accounted for by the increase of eosinophiles alone.

The following curves compare the time-relations of the eosinophilia in the present case and in the case of mouth infection which has been already dealt with[1]. The rise is rather earlier in the latter, but taking into consideration the fact that a slight increase of eosinophiles was present at the time of infection, there does not appear to be any necessarily significant difference in the two cases. The worm evidently begins to produce the substance to which the eosinophiles react at the

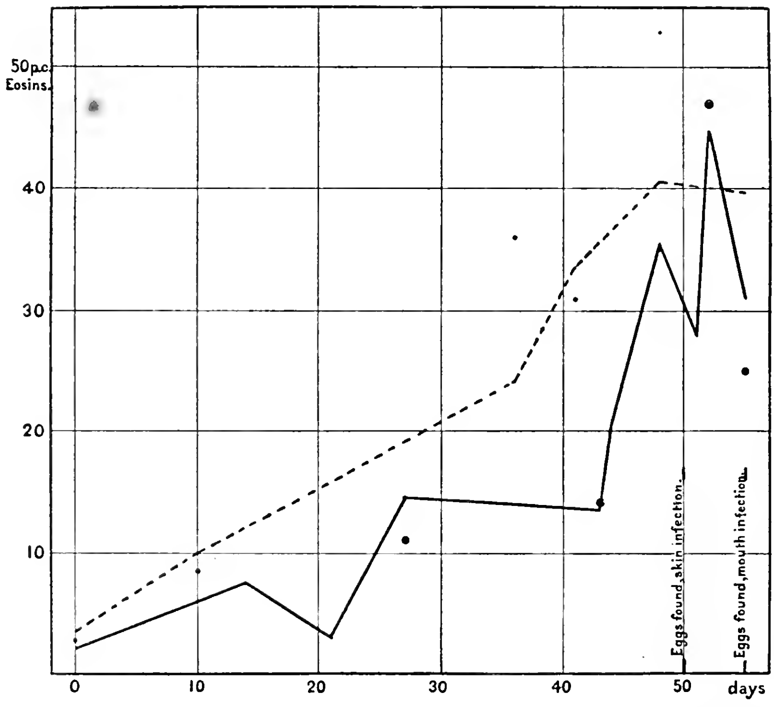


Fig. 1. The ordinates represent percentage of eosinophiles, the abscissae days. The continuous line is the skin, the dotted line the mouth infection. The separate points give the absolute eosinophiles where these were determined.

[1] This *Journal*, vol. IV. 1904, p. 462.

stage in its development which is reached 14 to 21 days after its entry into the body[1].

During the last eighteen months a number of important observations have been made on the skin infection in ankylostomiasis. Looss[2], working with *A. caninum* and dogs, has completed his demonstration by showing that the larvae leave the neighbourhood of the skin in the blood stream, pass through the right heart and are arrested in the lungs, whence they travel up the trachea and down the oesophagus to the intestine. These results have been fully confirmed by Schaudinn[3], who, using *A. duodenale* and monkeys (*Inuus*), found larvae in the blood of the right heart and in the lungs a short time after infection, and adult worms in the intestine from a previous application of larvae to the skin. C. A. Smith[4] has brought about skin infection in man with *A. americanum*; he found eggs in 45 days. This period, as well as that of 50 days found in the present experiment, is much shorter than that previously found by Looss (71 days), and by Pieri (71 days), and is so little different from that observed in mouth infections that any emphasis which has previously been laid on the difference seems to be hardly valid. With *A. caninum* and dogs, Lambinet[5] has shown that eggs appear in the stool in just the same time (20 days) whether the larvae are swallowed, or applied to, or injected under, the skin. Herman[6] has also successfully infected himself by the application of larvae to the skin.

The clinical aspects of the skin eruptions and their essential connection with the disease have been fully described by C. A. Smith[7] in the United States ("ground itch"), and by Ashford, King, and Igaravidez[8] in Porto Rico ("mazamorra"). The natives in the latter

[1] The eosinophilia is extraordinarily persistent. A man who while infected showed 17–19 p.c. still has 11 p.c. though it is more than 30 months since treatment was commenced and fully 20 months since he was restored to health and his stools found to be free from ova on repeated examinations.

[2] Communicated on behalf of Prof. Looss by Prof. Elliot Smith to the meeting of the British Medical Association at Oxford; see abstract in *The Mining Journal*, Aug. 6, 1904, p. 138.

[3] *Deutsche med. Wochenschr.* 1904, p. 1338.

[4] *Journ. Amer. Med. Assoc.* vol. XLIII. 1904, p. 592.

[5] *Bull. de l'Acad. Roy. de Méd. de Belgique*, Jan. 28, 1905. F. Smith, *Journ. Roy. Army Med. Corps*, vol. IV. 1905, p. 335, and H. Liefmann, *Zeitschr. für Hygiene*, vol. L. 1905, p. 349, have also recorded successful skin infections in dogs with *A. caninum*.

[6] *Acad. Roy. de Méd. de Belgique*, procès-verbal, Jan. 28, 1905, p. 8.

[7] *Journ. American Med. Association*, Sept. 19, 1903.

[8] *Report on Anaemia in Porto Rico*, San Juan, 1904. This valuable report shows

place have long recognised the aetiological connection between mazamorra and anaemia, just as in Cornwall the miners knew that dyspnoea followed "New sump bunches." There is an older account of ankylostomiasis which is of especial value and interest in this connection[1]. The author, after a careful review of the epidemiology of the miners' anaemia of the French coal-field, concluded at the time of writing that the anaemia is due to products of the distillation of heated coal. No suggestion had then been made of a parasitic origin. He describes however certain skin affections as an essential part of the disease, which are obviously identical with those seen in Cornwall. They were celebrated in the topical songs of the period and consisted of: "(1) a papular-vesicular-pustular eruption, very painful, called *ampoules,* affecting the hands and feet, and, in a more generalised form, the parts of the body coming in contact with the mine-water and coal-dust. (2) An urticaria, called *gourmes,* forming red indurated swellings up to the size of a bean, extending into the subcutaneous tissue. Each lump lasts 2 or 3 days, and there are successive crops[2]. They affect parts which have been in contact with wet coal and appear 12 to 24 hours after contact. When the urticarial nodules are numerous, they are accompanied by severe bronchial catarrh (*catarrhe des gourmes*)." This last observation is of great interest in view of the recent demonstration that the lungs form a temporary lodgement for the larvae on their way from the skin to the bowel. Skin symptoms do not appear to have been noticed in the Belgian and Westphalian coal-mines.

in a striking manner the fearful havoc which *Ankylostoma* can play in a bare-footed and untreated population. A large proportion of the natives seem to have less than 50 p.c. haemoglobin.

[1] A. Manouvriez, *De l'anémie des mineurs, dite d'Anzin*, Valenciennes, 1878. See also *De l'anémie ankylostomiasique des mineurs*, Valenciennes, 1904.

[2] It has been previously pointed out (This *Journal*, vol. III. 1903, p. 109), that attacks of urticaria, like the general pruritus, may also occur long after the patient is removed from any possibility of fresh infection. See also *Journ. Roy. Army Med. Corps*, vol. IV. 1905, p. 652.

PILOCARPINE AND OTHER REAGENTS IN RELATION TO PRECIPITIN IMMUNITY.

By O. INCHLEY, M.A., M.D. (Cantab.).

[Thesis for the Degree of M.D., University of Cambridge.]

(*From the Pathological Laboratory, University of Cambridge.*)

Introduction.

It is generally recognised that, in certain diseases, recovery mainly depends upon the prompt and efficient reaction of the tissues forming antibodies, such as bacteriolysins, antitoxins, and the like, and it is possible that these peculiar biological responses may be influenced by the administration of drugs.

The problem is open to direct experimental investigation. For this purpose it is necessary to select one of those reactions of immunity which will lend itself to easy quantitative estimation. It was suggested by Dr Nuttall that the precipitin reaction would conveniently suit this purpose, and that the action of pilocarpine should be first investigated.

This suggestion was based upon the work of Salomonsen and Madsen[1] in the case of antitoxin production. These authors found a large and rapid increase of antitoxin in the serum of the horse immunised against diphtheria toxin; this increase occurring after the intravenous injection of pilocarpine. They found that this increase in the antitoxic power of the serum occurred at the time when the horse was showing the obvious physiological effects of the drug. They remarked that, when the doses of pilocarpine were repeated the effect upon the antitoxic strength of the serum became less with repeated doses. And they also noted that the increase in antitoxin after pilocarpine could best be elicited when the animal was naturally decreasing its yield of antitoxin.

[1] Cited by Nuttall, *Blood Relationship and Immunity* (Cambridge, 1904).

The precipitin reaction is suitable for this kind of investigation, the technique being comparatively simple. It is obvious that, should the effect of pilocarpine with precipitins be like that already described with antitoxins, a confirmation of some importance is obtained.

There is a certain ambiguity in the meaning of the term "*the precipitin reaction.*" When discussing pathological processes we speak of the inflammatory reaction of the tissues; and in a similar vague manner this term is used to express the biological processes concerned in the formation of precipitins in the immunised animal. This formation of precipitins is a biological response, and will be termed the "*precipitin response.*" The "precipitin reaction" will then be used in the chemical sense concerning events in the test-tube; thus the "precipitin reaction" is that between serum and antiserum which results in the deposit of a precipitum. The term "precipitum" is technically limited to this particular form of precipitate. Thus the "precipitin reaction" is an observable phenomenon, the "precipitin response" is a biological process of which we know nothing except by inference from observed precipitin reactions.

If a rabbit be taken and subjected to a series of injections of serum, the serum being obtained from an animal of a different species, the blood of this rabbit in process of time acquires a new property. For if the serum of the rabbit so treated be mixed in the test-tube with the serum such as was used for injection, a cloudiness gradually appears in the mixture. As the cloud increases, its uniformity gives place to a granular appearance; the finer particles then become aggregated into small discrete masses, which finally sink to the bottom of the test-tube, leaving the fluid above quite clear.

The serum of the rabbit which has acquired this specific property is called an "antiserum" to the serum with which it reacts. Such an antiserum is regarded as containing a substance called "precipitin" which combines with the "precipitable substance" in the serum, the mutual neutralization being evidenced by the appearance of the cloud of "precipitum."

The course of the biological response in the tissues of the rabbit may be gauged by daily estimations of precipitin in samples of blood taken from the rabbit, the quantity of precipitum formed being estimated volumetrically.

The question arises whether, other conditions remaining fixed, the quantity of observed precipit*um* is proportional to the amount of hypothetical precipit*in*. No systematic observations on this point have yet

been published. It is possible that the law which formulates the relation of toxin to antitoxin will be found to apply in the case of the precipitins also.

So far as the precipitin reaction is concerned, it has been shown that, after the precipitum has become deposited from the mixture, free precipitin and precipitable substance may be found in the supernatant fluid. Hence if a further addition of one or the other be made, a fresh deposit of precipitum may be obtained. Let us suppose that with a definite mixture of serum and precipitating antiserum there results *in the end* a definite quantity of the combination, "precipitin-precipitable substance." Now is the precipitum which we see the same in quantity as the "precipitin-precipitable substance" which results from the reaction? does all the precipitum "come down"? Nuttall has found that, by adding antiserum drop by drop to undiluted serum, the evanescent precipitum observed at the moment the drop is added, redissolves or disappears in the excess of serum present; the precipitum is said to be soluble in excess of serum. Does the "precipitin-precipitable substance" here dissociate into precipitin and precipitable substance; or does the substance remain in combination but mechanically dissolved in excess of serum?

There are experiments which suggest that the precipitin reaction is reversible. It is true that toxins deteriorate on keeping, and become, as we say, converted into toxoid. It is also true that precipitating antisera can by heating be inactivated. In this condition the power of specific union with precipitable substance is retained, while the property of bringing about the clouding is lost. Müller regards the combining antibody in inactivated antiserum as "precipitoid"; in Ehrlich's terminology this would be spoken of as precipitin in which the zymophoric group had been destroyed. If then precipitable substance be mixed with such "precipitoid" a soluble compound is formed, so that, on now adding precipitin, there is no available precipitable substance with which the precipitin may combine. No precipitum is formed; the precipitoid prevents the precipitating action of precipitin when the latter is added later to the mixture. So far we may consider precipitoid to have a strong avidity for precipitable substance, so that precipitable substance no longer exists, and there is nothing for precipitin to combine with. But the following experiment, due to Eisenberg, may be performed. The reaction is that between precipitable substance, precipitoid, precipitin, and their compounds when mixed together. First definite quantities of precipitable substance and precipitoid are

mixed together, and it is found, as before, that on adding a certain amount of precipit*in* no deposit of precipit*um* takes place; the experiment is then repeated in a series, the amount of precipit*in* added being each time increased. It is then found that when a sufficient quantity of precipitin is present a deposit of precipitum appears. Here then the combination of precipitoid with precipitable substance must have been dissociated, the precipitable substance so freed then combined with precipitin, the product becoming evidenced by a deposit of precipitum. Thus the reaction appears to be reversible and largely dependent upon the relative masses of the reagents present.

There is evidence to show that the same general principles apply with regard to the allied haemolytic phenomena which involve the reactions between red cells, specific immune body, and complement.

Finally the question arises in the case of the precipitin reaction: is the precipitum which we see and whose volume may be measured, itself the combination "precipitin-precipitable substance"? or may it be that this product of the reaction is always invisible to us, but by a secondary action precipitates other substances present in the serum, and so causes the cloud of precipitum? Obermayer and Pick separated the different constituents of egg white and injected them severally into animals in order to produce corresponding antisera. These observers were surprised to find that one substance might in the animal lead to the formation of a precipitin, which did not react with this substance, but with some other constituent of egg white. It may also be remarked that pure crystallised egg albumin was quite incapable of exciting any precipitin response at all.

With regard to the precipitin reaction, it is clear that no definite statement as to the quantitative relation of precipit*um* to hypothetical precipit*in* can be made. There is first the quantitative relation of precipitin to "precipitin-precipitable" substance; and, secondly, the relation of the latter to the precipitum which we observe. The answer to these questions can be supplied by experimental investigation only. Moreover each precipitin reaction observed on injecting different sera may be the resultant of a number of precipitin reactions occurring together.

Methods.

It will be remembered that at the outset the precipitin response was distinguished from the reaction *in vitro*. It is the effect of drugs

upon the course of the precipitin response which we wish to investigate, and it becomes necessary here to define our position clearly.

Just as the temperature chart may be taken to indicate the course of a fever, so a chart may be obtained to indicate the course of the precipitin response in the animal yielding antiserum. Each day a few drops of blood are withdrawn from the animal's vein, and after clotting, the antiserum is obtained. A constant volume of the antiserum is then taken and tested by mixing it with a constant and definite quantity of the serum with which it gives the precipitin reaction. The amount of precipitum formed under these conditions is charted. Thus an arbitrary method of estimating precipitating power is fixed upon, while what is indicated on the chart is the precipitating power of a unit volume of blood from day to day. Putting aside the question of the exact relation of precipitating power of the sample to its precipitin content it is obvious that not only is the precipitating power of the sample dependent on the formation of precipitin by the tissues, but it is also dependent on the dilution or concentration of the blood itself from time to time.

Such precipitin charts serve the present purpose, for the daily readings mark out a curve sufficiently uniform to be taken as a normal, so that sudden and marked variations in the curve following the administration of the drug are clearly seen. It is, of course, essential that the daily precipitin estimations should be performed in an exactly similar manner, so that, whatever may be the complicated sequence of events in the reaction *in vitro*, still if the blood itself should vary uniformly from day to day the precipitin chart should also vary uniformly and map out a curve.

The following experiment shows the necessity of keeping the conditions uniform during the formation of the precipitum in the test-tube. In performing estimations of precipitating power it is usual to add a definite quantity of the antiserum to be tested to a larger quantity of fluid consisting of serum largely diluted with normal saline; here the antiserum naturally sinks to the bottom of the test-tube, so that the contents must be mixed together by inversion. When performing several estimations it is easy to fill in antiserum to a series of test-tubes containing the serum solution, and then afterwards to mix them up one after the other. In this case an initial and variable time has been allowed for events to occur at the zone of contact between antiserum below and serum solution above. It is obvious that in this zone the relation of the masses interacting is very different from that

which comes into existence when they have been shaken up together. When the contents are not shaken up the cloud of precipitum after a time makes its appearance in this region, and it might be supposed that in any case the subsequent mixing up would equalise matters. This, however, does not appear to be the case always, and it becomes necessary as an invariable routine to mix up the reagents thoroughly immediately on adding the one to the other.

Two similar estimations were performed in test-tubes *A* and *B*; like quantities of the same antiserum and serum solutions were used in both. The antiserum was a weak one, the precipitation occurring very slowly. In the case of tube *A* two clear days were allowed to elapse without shaking up the contents, but the other tube, *B*, was mixed at once. *A* was then shaken up, and the tubes allowed to sediment completely. Quantitative measurements of the deposits showed that *A* contained 5·7 c.mm. of precipitum, while *B* contained ·9 c.mm. only. Thus, in the zone of contact, in *A*, a considerable amount of precipitum was formed which on subsequent mixing was not redissolved. The matter was not further pursued, as the purpose was only to find a uniform method of procedure, and in consequence it became a routine practice to mix the contents immediately after adding antiserum to serum solution.

Not only in order to obtain a uniform curve on the precipitin chart must this method of estimation be a uniform routine, but the animal giving the response must be kept under uniform conditions also. It was found that the precipitating power of the sample of blood was affected by the state of digestion of the animal at the time when the sample of blood was taken. This of course might be an ultimate effect of assimilation upon the metabolism concerned in producing antibody; on the other hand it might be due to dilution or concentration of the total quantity of blood so affecting the power of the antiserum. To avoid this source of variation the sample was drawn in the morning before feeding; and the rabbits were kept in cages bedded with saw-dust, and fed at a definite time upon turnips.

The method of measuring precipitum which was used in this work, as well as the method employed for estimating the specific gravity of the blood, have been described elsewhere[1]. It is only necessary here to describe the method of preparation of the animal, and then to indicate briefly the routine of the precipitin estimation.

[1] Nuttall and Inchley (1904), this *Journal*, IV. No. 2; Inchley (1904), *Journ. of Physiol.* XXXI.

A rabbit was taken and subjected to a series of injections of hippopotamus serum; this serum had been previously heated to 55° C., thereby doing away with the normal haemolytic effects when injected. The animal received 1 c.c. of the serum into the ear vein, two clear days being allowed to elapse between each injection.

During the course of the ensuing precipitin response a few drops of blood were obtained daily from the ear; the sample was received into sterile Petri dishes, the serum separated, and its precipitating power estimated in the manner to be described.

When dealing with drugs which affected the distribution of leucocytes, a leucocyte count was made in the ordinary way, but no differential count was attempted.

Concentration of the blood.

The concentration of the blood was determined, when necessary, by estimating at the same time the specific gravity of the sample. On other occasions the depth of colour only was recorded by means of Oliver's haemoglobinometer, this test being likewise used to determine fluctuations in concentration. It is evident that concentration or dilution of the blood as a whole would cause an alteration in the result obtained with the precipitin test.

Thus, should any alteration in the precipitating power of the sample be observed, it becomes necessary to determine whether altered concentration is sufficient to account for the change. If neither this nor alteration in the number of leucocytes in the sample will explain the result, then presumably it is due to some deep-seated effect upon metabolism.

The precipitin estimation.

A standard dilution of hippopotamus serum was made, 1 part of serum being added to 20 parts of sterile normal salt solution. In each estimation a definite quantity of this was used as a reagent. ·5 c.c. of this solution was taken, and to it was added ·1 c.c. of the antiserum to be tested.

When an animal is treated with a foreign blood this can be shown to persist in the animal's circulation for a variable period by tests made with an antiserum appropriate to the foreign blood injected. Thus, if hippopotamus serum is injected into a rabbit, the presence of hippo-

potamus serum in the rabbit's circulation can be demonstrated for some time by the use of anti-hippopotamus serum. In carrying out such tests I diluted the antiserum 1:5 in saline before adding to it the serum to be tested.

The routine of each estimation occupied six days, consequently the state of the animal giving the precipitin response was not known till about a week later.

The order of procedure was as follows:

1st day—15 drops of blood were collected as a sample, and the antiserum allowed to separate from it.

2nd day—the precipitin reaction performed, ·5 c.c. of hippopotamus serum dilution was transferred into a small test-tube, and to this was added ·1 c.c. of the antiserum, and the contents then immediately mixed together.

3rd day—the precipitum which had now sedimented was transferred to the capillary tube.

4th day—in the capillary tube the column of precipitum was broken up by means of a horse-hair introduced from above and twirled about.

6th day—the height of the column of precipitum was measured and recorded.

It was found possible to avoid bacterial growth by ordinary precautions. When not in use the capillary tubes were washed through with tap water, the interior being easily cleaned with a pipe brush the bristles of which had been cut short for the purpose. The tubes were then kept immersed in chloroform water. In the precipitin charts which follow there is recorded either the height in millimetres of the column of precipitum, in which case a definite set of capillary tubes of uniform calibre was specially used, or the volume of the precipitum in cubic millimetres, this being calculated from the calibration of the tube. In the later curves the former method was employed.

The following drugs were employed: *Pilocarpine* was given in doses of 2–4 mgs. dissolved in saline solution and injected into the ear vein or hypodermically. *Turpentine*, a 1 % emulsion in saline was prepared, and injected subcutaneously in doses of 1 c.c. *Cinnamate of Soda*, 1 c.c. of a 4 % solution in saline was injected under the skin. *Quinine*, 1 c.c. of a 4 % solution of the chloride given subcutaneously. *Nuclein*, a 5 % solution of nucleinic acid from yeast, the dose injected being 1 c.c. of the solution. *β tetra-hydro-naphthylamine*, 5 c.c. of ·5 % solution in saline injected subcutaneously.

(1) *Action of pilocarpine.*

The effect of subcutaneous injections of pilocarpine upon the course of the precipitin response is shown in the accompanying chart (fig. 1).

In this experiment the rabbit received eight primary injections of hippopotamus serum; the chart shows the precipitating power of the serum above, while below is shown the disappearance of precipitable substance from the blood. The experiment covered a period of 76 days. The gradual onset of the response and increased facility of dealing with the foreign serum is observable. Towards the height of the response on the 28th day a subcutaneous injection of 2 mgs. of pilocarpine nitrate was made, and a corresponding increase in the precipitating power of the serum is shown on the chart. Similar but more marked effects are to be seen later, when the response was diminishing. It will be noted that the 8th injection of hippopotamus serum was given on the 22nd day, and that the decrease in precipitating power followed after the 31st day. In each case the sample of blood was taken half-an-hour after the injection of pilocarpine, so that of course the maximum attained is not necessarily the reading which is marked as maximum upon the chart. On the 54th and 55th days the injection of 2 mgs. of pilocarpine was followed by a distinct increase of precipitating power. On the 58th day three such doses were injected at intervals of half-an-hour and the sample of blood was taken half-an-hour after the last injection; in this instance practically no increase was observable.

The same chart also shows towards the end (beginning 61st day) the effect of further injections of hippopotamus serum; this however will be considered later.

It is to be remarked that the pilocarpine does not appear to have any permanent effect upon the curve. Within an hour or two at most the blood has returned to its condition prior to the experiment. Consequently, when the slow disappearance of the response as a whole is considered, such a rapid oscillation of the curve after pilocarpine is opposed to the view that this drug had stimulated the cells to secrete an additional quantity of precipitin into the blood, for it would seem reasonable to expect that the maximum would be maintained for some time at least.

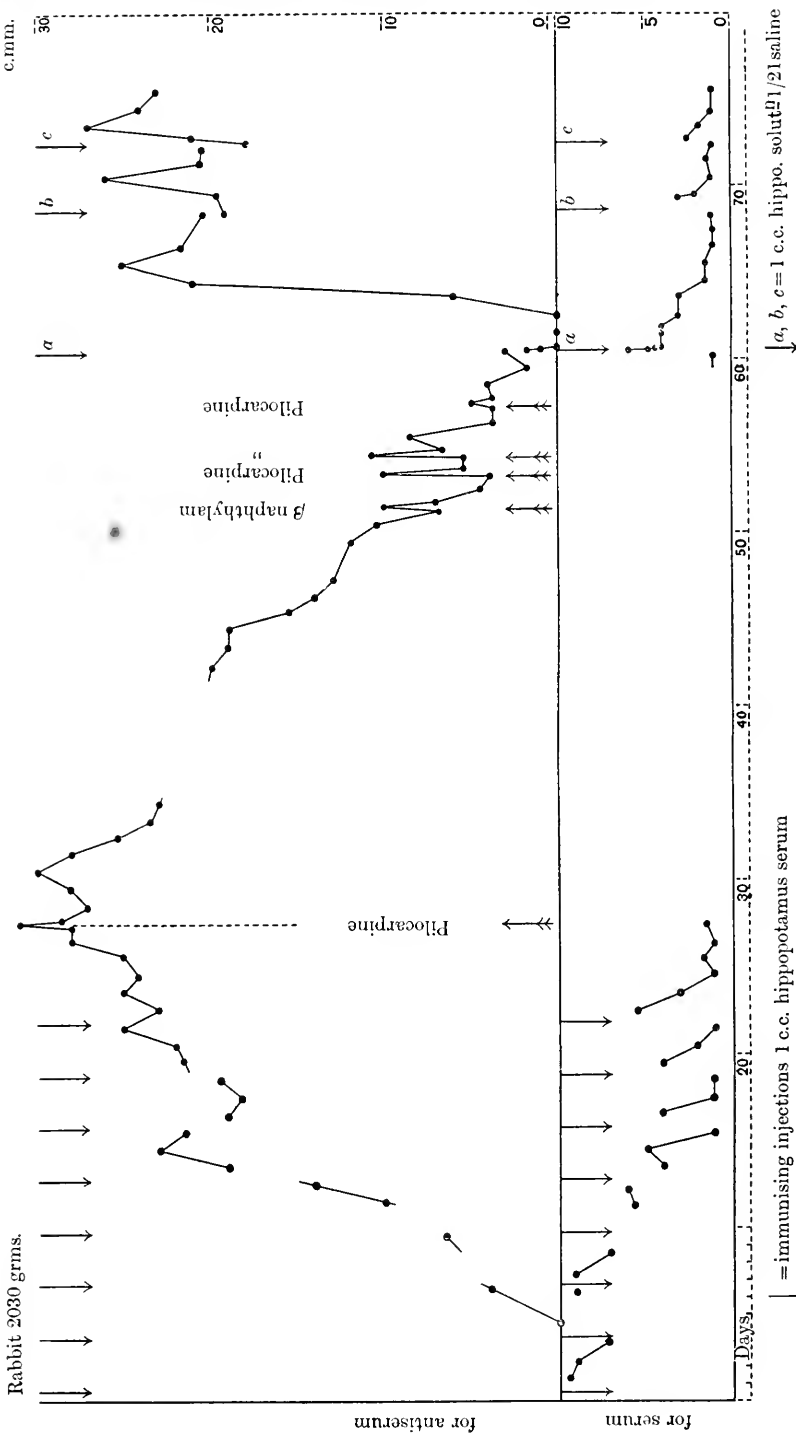

c.mm.
Rabbit 2030 grms.
Pilocarpine
β naphthylam
Pilocarpine
Pilocarpine
a
b
c
Days
for antiserum
for serum
= immunising injections 1 c.c. hippopotamus serum
a, b, c = 1 c.c. hippo. solutn 1/21 saline

Fig. 1.

Concentration of blood.

On the supposition that these effects might be simply due to concentration of the blood, the haemoglobin percentage was observed as each sample of blood was taken. This variation acted as an index of concentration. The close correspondence between the haemoglobin curve and that of the precipitating power is shown in the following figure (fig. 2).

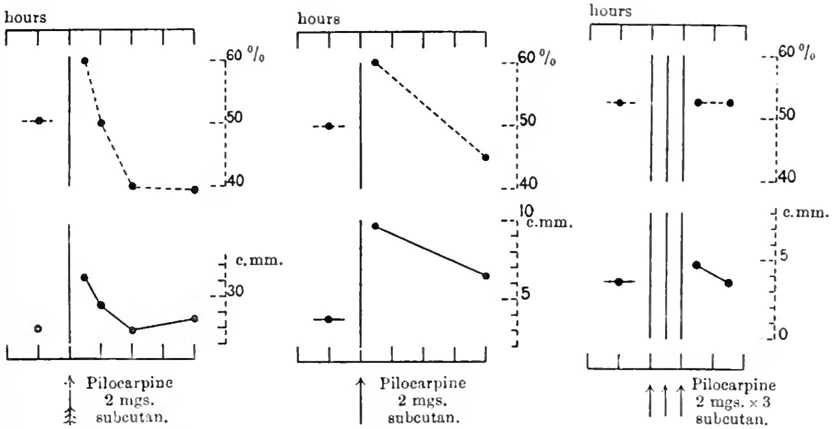


Fig. 2.

In these figures the precipitum is shown below, while above is represented the corresponding variation in the haemoglobin percentage. They show in greater detail the experiments indicated in the general chart (fig. 1) on the days 28, 54, and 58.

As the haemoglobinometer is calibrated not for rabbit but for human blood, it was necessary to employ some other method of estimating the amount of concentration. The experiment was consequently repeated, the specific gravity of the blood being observed. The haemoglobin estimations show that a diminution in the volume of the plasma had occurred. A simple increase in specific gravity alone might possibly be attributed to the addition of heavier bodies to the plasma.

In the following chart (fig. 3) the effect of an intravenous injection of 4 mgs. of pilocarpine is shown.

This curve was obtained from the same rabbit as before, after an interval of three weeks, the animal being again treated with hippopotamus serum. Fifteen minutes after the injection of pilocarpine the precipitating power had risen from 19·3 to 30·6 mm., and there was

a corresponding alteration in the specific gravity of the blood from 1049 to 1055.

Three days later the same dose was administered again, this time subcutaneously. The sample of blood was taken half-an-hour after this, and a very slight effect on the precipitating power was observed; as before pointed out, the negative result may be simply due to the sample

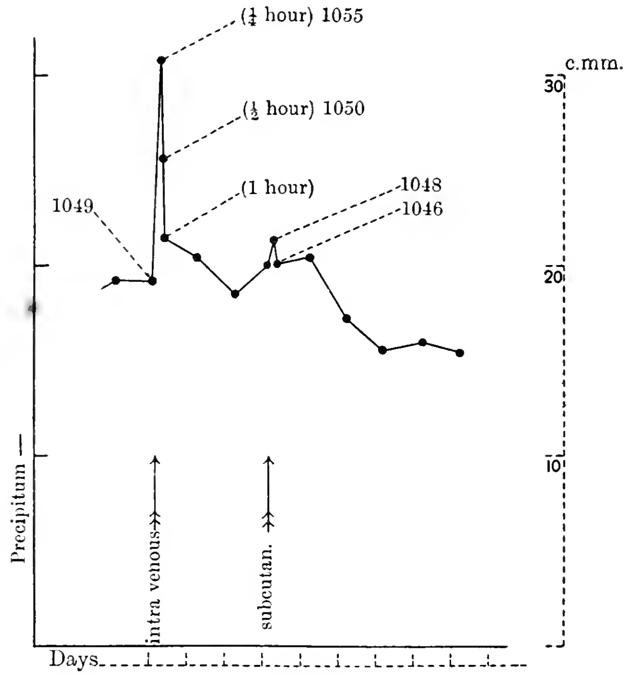


Fig. 3.

not being taken at the right time. But it is to be noticed here that with a slight alteration in precipitating power there is a corresponding slight alteration in the specific gravity of the blood.

In the first case then there was an increase of 50 % in precipitating power, while the density of the blood was altered from 1049 to 1055. This alteration in density is equivalent to the abstraction of about 12 % of water[1]. If the fluid removed be of greater density than water it is

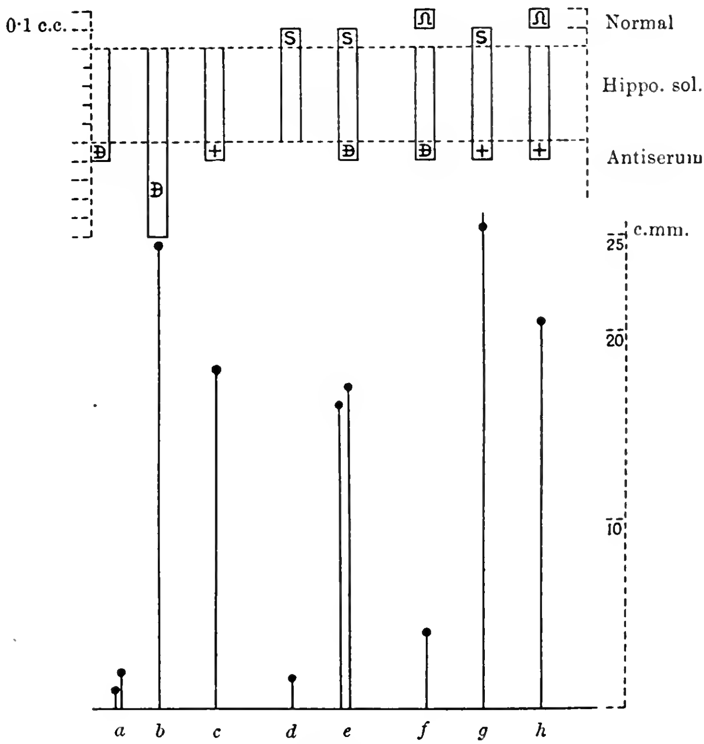
[1] Let x be the number of c.c. of water added to 100 c.c. of blood with sp. gr. 1055, so that the mixture $(100 + x)$ c.c. has now a sp. gr. of 1049;

$$\text{then } (100 \times 1055) + (x \times 1000) = (100 + x)\,1049;$$

$$\therefore\ x = 12.$$

obvious that a larger volume percentage would have to be abstracted in this case. If then the concentration of the blood be only 12 %, how is the increase of 50 % in precipitating power to be accounted for?

The following figure shows diagrammatically the effect produced upon the precipitating power by varying the amount of antiserum in the mixture.



Ω = normal rabbit serum.
S = serum of rabbit 60th day, fig. 1.
+ = stock antiserum.
Ð = stock antiserum dilut. $\frac{1}{5}$ normal saline sol.

Fig. 4.

Here the mixture of serum and antiserum is represented above, while the amount of precipitum which resulted is shown below.

(*c*) indicates that 18 c.mm. of precipitum were obtained when 0·1 c.c. of *stock antiserum* was added to 0·5 c.c. of *hippopotamus* serum dilution.

In (*d*) the same proportions were employed, only 0·1 c.c. of *a very*

weak antiserum was used instead of the stock antiserum. Here only 1·5 c.mm. of precipitum were obtained.

In (*g*) a further experiment was performed, in which equal parts (0·1 c.c. of each) of both strong and weak antisera were present. It is observed that 26 c.mm. of precipitum now appeared.

Thus with a constant quantity of the precipitable serum solution, a powerful antiserum gave 18 c.mm., a weak one only 1·5 c.mm., while the two together gave as much as 26 c.mm. So that a very slight increment in precipitating substances apparently led to an increase of 50 % in the precipitum deposited in this case.

It may be observed that the stock antiserum here used gave 18 c.mm. of precipitum, while the serum of the rabbit in which the action of pilocarpine was tried gave 19 c.mm. So that the two antisera were of approximately equal strength.

Next the stock antiserum was diluted with normal saline, a dilution of one in five being obtained.

In (*a*) and (*b*) the same quantity (0·5 c.c.) of precipitable serum dilution was again employed, but in the first case 0·1 c.c. of dilute antiserum was added, while in the second case 0·5 c.c. was taken. It will be seen that five times the amount of antiserum present roughly results in about 25 times the amount of precipitum deposited.

If this be taken as roughly indicating the relationship with these solutions, it follows that a 50 % increase in the amount of precipitum deposited would be accounted for by a 10 % increase in precipitating substances present. And this corresponds with the 12 % concentration in the blood which was observed after pilocarpine administration in the last experiment. In other words, the mere concentration of the blood after pilocarpine appears to be amply sufficient to account for the increased quantity of precipitin deposited.

We have therefore reason to believe that the increased precipitating power in the serum which follows pilocarpine is a result of the concentration of the blood which it brings about. Such a view is also in accordance with the known pharmacological action of pilocarpine.

The drug has a specific stimulating effect upon the nerve terminations in glands and unstriped muscle. It leads to increased peristalsis and diarrhoea, and causes shrinkage of the spleen and organs supplied with involuntary muscle fibres. In this connection it may be mentioned that electrical stimulation of the spleen in leukaemia has been found to bring about an immediate increase in the leucocytes in the blood. That pilocarpine leads to a leucocytosis in the normal animal is a state-

ment in which there is not entire agreement. Ewing was unable to obtain this effect in rabbits. Its action upon the terminations of secretory nerves induces a copious general secretion throughout the body, and in this way a considerable loss of fluid occurs through the skin, lungs, salivary glands, etc.

So far as is known, the only glandular tissues which are induced to secrete by the administration of pilocarpine are those having a nervous control, yet a nervous mechanism for the production of antibodies would seem at any rate a most unlikely one.

(2) *Action of nuclein and of fowl sera.*

The following chart records the precipitin response during which the animal received injections of yeast nuclein, and also of fowl serum, that is to say, of a serum different from that which was originally employed to excite the precipitin response.

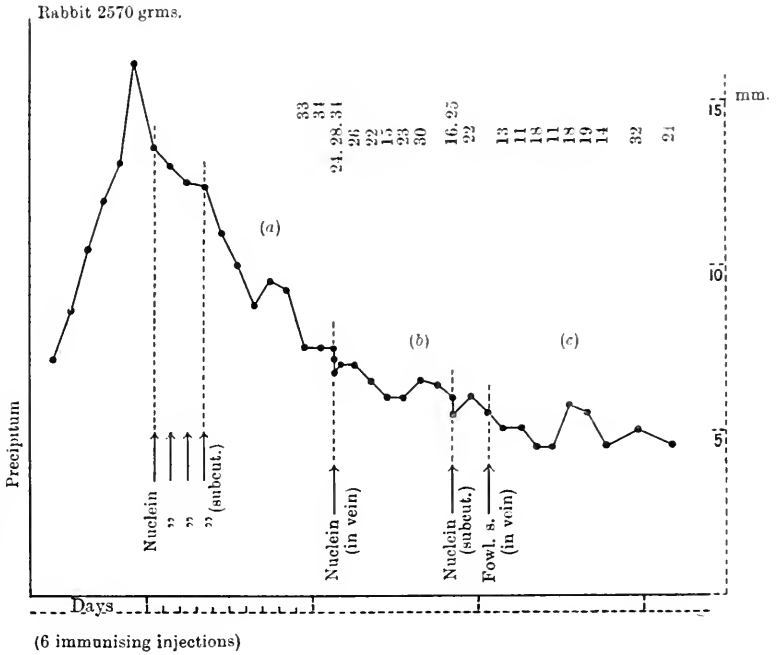


Fig. 5.

It is to be seen that after the injection of fowl serum the curve at first continues its descent apparently unaffected by the treatment; on the 5th and 6th days however a slight rise is recorded at (*c*). This same

serum tested with fowl serum for the presence of antifowl gave no precipitum, showing absence of specific antibody to fowl.

If now a reference be made to fig. 1 it will be found that a further injection of exciting serum (*a* in fig. 1) led to a second precipitin response which reached its maximum also on the 5th day after the injection. And a similar effect is also shown in fig. 6.

As a series of injections of fowl serum would lead to an antifowl response, the interesting question arises whether the injection of fowl serum in the experiment recorded led to a slight stimulation of the mechanism concerned in the anti-hippopotamus response. To produce a modification in the response other than by its specific exciting serum, the most likely reagent would presumably be a serum of another animal, unless an entirely different sequence of events underlies each precipitin response.

Five days after injections of yeast nuclein still smaller fluctuations in the curve were observed (*a*) and (*b*). These however are so slight as to be within the region of experimental error.

It is manifest that no appreciable effect is produced upon the curve by these agents. That the biological mechanism for the production of antibodies is affected only by specific substances, is in accordance with what is known of glandular mechanisms in general. Drugs which markedly affect secretion usually do so by their action upon the endings of nerves supplying the gland: and conversely we are beginning to see that glands, like the pancreas, which are not coordinated for rapid responses to altered environment, seem to respond to definite physiological substances and to them only.

(3) *Effect of further injections of exciting serum and of various drugs.*

The effect produced by further injections of exciting serum is shown in the later part of the first chart, fig. 1, *a*, *b*, *c*: 1 c.c. of a 1 : 21 hippopotamus serum dilution was injected at a time when the primary precipitin response had almost disappeared.

Here the dose was 1/20 of that used in the primary exciting injections. There is a rapid ($\frac{1}{2}$ hr., 1 hr., 3 hrs. samples) disappearance of precipitin and a corresponding diminution in free precipitable substance in the blood after receiving the injection; on succeeding days there is then a more gradual disappearance of precipitable substance, while precipitin begins to appear on the third day and rapidly rises to a maximum on the fifth day after the injection. It

may be observed that although the dose was very small a high maximum was attained.

At *b* and *c* similar injections were repeated. The sample taken one hour after injection *b*, showed a slight decrease in precipitum, the diminution after injection *c* is that shown by a sample taken $\frac{1}{2}$ hour after the injection. An interesting point to be observed is that the time between the injection and the resulting maximum gets shorter with each injection. Thus after the second injection *b* the maximum is attained on the second day, after the third injection it is reached on the day following the injection. Apparently the previous precipitin response leaves the animal with a mechanism peculiarly susceptible to the exciting serum.

A rabbit was next taken which had given the precipitin response five months previously, 1 c.c. of a 1 : 21 hippopotamus serum dilution was injected intravenously; examinations of the blood showed that no response occurred.

Next two rabbits of approximately equal weight were both treated in a similar manner with six immunising doses, each of 1 c.c. of undiluted serum. The one rabbit *A* had not before been treated with serum; in the other *B* the precipitin response had been induced five months before.

Fig. 5 is the chart obtained from the previously normal animal *A*, while fig. 6 is that of the other one, *B*. It is seen that a much greater response occurred in the one which had previously been treated.

Apparently after the primary response has subsided, there remains for some time a potential mechanism sensitive to the particular stimulus which had been employed, and to which the response owed its origin. Possibly the active formation of antibody during the course of the primary response has given a bias to the cell metabolism, leaving a kind of cell-memory so far as that stimulus is concerned, which in its turn endures for a certain length of time.

In figs. 1 and 6 are recorded certain observations upon the disappearance of foreign serum from the blood. It was found that, after an injection had been made, there followed a gradual disappearance of precipitable substance from the blood. In neither case, however, was the disappearance observed to be complete; towards the end of the response a trace was still obtained.

In view of the work on the nature of the reaction already quoted, it is possible that the antibody in the blood does not completely neutralize the exciting substance; in other words, the process of antibody formation

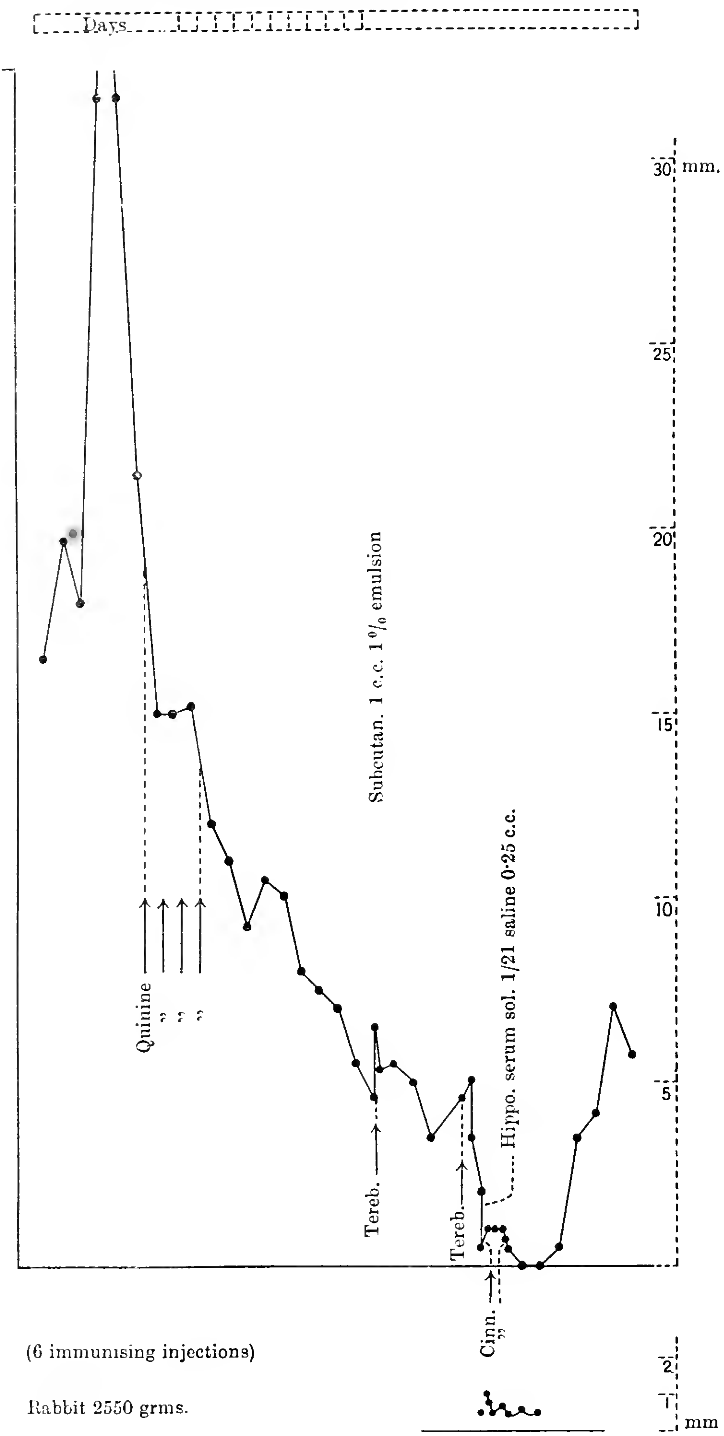

Days
30 mm.
25
20
15
10
5
2
1
mm
Subcutan. 1 c.c. 1 % emulsion
Quinine
,, ,, ,,
Tereb.
Tereb.
Hippo. serum sol. 1/21 saline 0·25 c.c.
Cinn.
,,
(6 immunising injections)
Rabbit 2550 grms.

Fig. 6.

goes on till a residue remains which is tolerated by the tissues, or until the stimulus becomes so small as to be insignificant. The question is an interesting one, only to be solved by further investigation.

Fig. 6 also shows slight irregularities in the course of the curve, which may perhaps be caused by the drugs injected. Thus with quinine injections the disappearance of precipitating power appeared to be less rapid, and 5 days after the last injection a slight increase is observed. Subcutaneous injections of oil of *turpentine* emulsion led to a small and temporary rise in precipitating power, like that observed with pilocarpine. Towards the end of the curve, a small injection of the primary exciting serum was again administered, after an interval of a few days a distinct rise occurred similar to that described in fig. 1. Four hours after this injection a mere trace only of precipitum was obtained. *Cinnamate of Soda* (1 c.c. 4 %) was then administered subcutaneously; the slight alteration in the readings observed is however again too small to be considered.

Conclusions.

As a result of these researches, the general conclusion arrived at is that the processes concerned in the elaboration of specific antibodies are not appreciably affected by these drugs. Where a temporary increase follows the injection of the drug it is probably explainable in other ways. Thus in the case of pilocarpine the concentration of the blood, presumably the result of the general glandular activity, is sufficient to account for the increase in the precipitating power observed.

In conclusion I have to acknowledge my deep indebtedness to Dr Nuttall, F.R.S., throughout; and I have also to thank Dr Dixon of the Pharmacological Department for his advice in the selection of the drugs.

AN IMPROVED METHOD OF CALCULATING BIRTH-RATES.

PART II. RESULTS.

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In the last number of the *Journal of Hygiene* we described an improved method of calculating birth-rates, by means of which exact correction can be made, in comparing the birth-rates of two communities or of the same community at different periods, for variations in the proportion of married women at childbearing ages, and for the different fertility-rates at different ages of childbearing life. The value of this method consists in the fact that by its means true differences in childbearing can be discovered, the differences due to variations of age or marital condition being eliminated.

In this paper it is proposed to illustrate these points by examples taken chiefly from the official statistics relating to the counties of England and Wales, leaving detailed tables and the discussion of more extended data for publication elsewhere. Throughout this paper, the termination *shire* is omitted from the name of each county.

The following examples compare the English population in 1881 with that in 1901 or 1903. The year 1881 has been chosen, partly because of its convenience in being a census year, chiefly because it represents a point very near the year 1876 when the highest recorded birth-rate in England and Wales occurred.

The subject can best be approached in stages, each illustrating an important aspect of the corrections required in ascertaining the true value of crude birth-rates.

(1) *Changes in the proportion of women aged* 15—45 *in the population.*

In the year 1881 the number of women aged 15—45 per 1000 of the total population was 230·6; in 1901 it had increased to 249·7. This result would naturally follow from the steady lowering of the birth-rate since 1876. In 1881 and in 1901 alike London had a higher proportion of women aged 15—45 in every 1000 of its population than any of the 40 counties, viz. 273·7 in 1901 and 261·7 in 1881. Worcester, Surrey, Lancashire, and Middlesex were the next highest in 1881, and Surrey, Middlesex, and Lancashire in 1901. The lowest proportion both in 1881 (196·9) and in 1901 (211·4) was in Huntingdon; Wilts, Salop, and Rutland being nearest to Huntingdon at both dates.

(2) *Changes in the proportion of wives among women aged* 15—45.

In 1881 Durham had the highest number of wives per 1000 women aged 15—45, viz. 579, Glamorgan coming next with 551, and Stafford third with 546. In 1901 Glamorgan had the highest number, 548, Monmouth coming next with 537, and Durham third with 534. At the bottom of the list in 1881 were Sussex 433, Surrey 423, Westmorland 422, and Cornwall 416; and in 1901 Hereford 418, Surrey 412, Sussex 391, and Westmorland 385.

In the above examples it will be seen that while the proportion of women of childbearing age to the total population has increased, the number of wives in every 100 women of these ages has declined. The same holds good for England and Wales as a whole, in which, while the proportion of women at the childbearing ages in the entire population has increased 8·3 per cent., the number of wives at these ages has declined 4·7 per cent. between 1881 and 1901.

It will be remembered that we are dealing throughout with legitimate birth-rates. The crude legitimate birth-rate of England and Wales was 33·0 per 1000 of population in 1871 and 27·4 in 1903. The crude illegitimate birth-rate was 2·0 in 1871 and 1·1 in 1903. We are not concerned in this paper with the decline in the illegitimate birth-rate, which is still greater than that in the legitimate birth-rate.

(3) *Comparison of standard birth-rates.*

The above data, although interesting and suggestive, do not determine the question whether the decline in the legitimate birth-rate between 1881 and 1901 has been partially, and if so, to what extent it has been caused by alterations in the proportion of wives in the population, and in the ages of these wives. We have still to ascertain by the method described in our former paper the changes due to altered proportion of wives, and to the varying fertility of these wives at ages 15—, 20—, 25—,...40—45.

The first step in this determination is to ascertain standard birth-rates, *i.e.* the birth-rates which would occur in each county assuming that the birth-rates of Sweden at ages 15—, 20—, 25—,...40—45, held good for the married women in each county at the corresponding ages. Standard birth-rates thus obtained are the only accurate index of the capability for a legitimate birth-rate possessed by the population of each county. The comparison of 1901 with 1881 in this respect brings out some important results. In 1881 Durham had the highest standard, *i.e.* potential birth-rate, 38·33 per 1000, London coming next, 37·66, Lancashire third, 37·34, and Nottingham fourth, 37·24; followed in order by Glamorgan, York, Worcester, Warwick, and Stafford. In 1901 at the upper end of the scale Glamorgan came first, 38·78, Durham second, 38·04, Nottingham next, 37·22, then Warwick, 37·19, followed in order by London, Essex, Middlesex, York, Lancashire, and Stafford.

At the lower end of the scale Cornwall had the lowest potential birth-rate in 1881, 26·67. It was ten places from the lowest in 1901 when it was 29·95. Rutland was next lowest in 1881, and lowest of all in 1901, being 27·10 in 1881 and 26·09 in 1901; Salop was 27·18 in 1881 and 27·45 in 1901. With the exception of Cornwall which is only partially so, the counties having the lowest potential birth-rates are nearly all of them purely agricultural counties.

Sixteen counties have a rather lower potential birth-rate in 1901 than in 1881. If the rate for 1881 be taken as 100, the greatest decrease was that for Hereford, whose proportional figure was 91 in 1901, and that for Worcester, which was 94 in 1901. Taking 1881 again as 100, the greatest increase was in Cornwall 112, in Essex 110, Middlesex and Monmouth 109, and Northumberland 107. Twenty-five counties showed some increase in their potentialities for a high birth-rate in 1901 as compared with 1881.

(4) *Comparison of crude legitimate birth-rates.*

Before applying the corrections which the standard birth-rates enable us to apply to the crude birth-rate, and before by this means separating the extrinsic or arithmetical changes from the intrinsic changes caused by alterations in fertility, it is convenient at this stage to make a preliminary comparison of the crude birth-rates of the counties in 1881 and 1901.

In 1881 Durham had the highest crude birth-rate, 37·88, Stafford coming next, 36·14, followed in order by Glamorgan, Lancashire, Nottingham, Derby, and Leicester. The lowest crude birth-rate in 1881 was in Hereford 25·44, the next lowest Salop 27·08, Cornwall 27·09, after which came in order Huntingdon, Westmorland, Dorset, Devon, Rutland, and Sussex.

In 1903 the crude birth-rate of every county except Monmouth (the crude birth-rate of which was 6 per cent. higher than in 1881) was much lower than the corresponding birth-rate in 1881. Taking the birth-rate in 1881 as 100, the counties in which there was the least decline in 1903 were Glamorgan 95, Northumberland 93, Salop 91, Durham 90; the counties in which there was the greatest decline being Rutland 69, Sussex 73, Oxford 74, Westmorland and Northampton 75, Bedford 76, Somerset 77, Berks and Devon 78. On the whole the rural counties have suffered from a decline of the legitimate crude birth-rate more than the industrial and urban counties.

(5) *Comparison of corrected legitimate birth-rates.*

Compare these results with the corresponding results when due correction has been made as already described. In 1881 the corrected legitimate birth-rate of England and Wales was 32·7 per 1000 of population; in 1903 it was 27·4, the comparative figures being 100 and 84. In 1881 the highest corrected county birth-rate was in Rutland 36·39, next came Cumberland 35·99, Cornwall 35·46, and Stafford 35·42. In 1903 the four highest were Monmouth 33·13, Durham and Salop 31·43, and Cumberland 31·13. The four lowest in 1881 were Worcester 30·43, Hereford 30·83, London 30·92, and Yorks 31·34. In 1903 the four lowest were Sussex 24·15, Devon 24·28, Northampton 24·59, and Bedford 25·09. The significance of the changes produced by the correction will be more clearly seen when they are stated in tabular form:

BIRTH-RATES.

	1881		1903	
	Crude	Corrected	Crude	Corrected
Durham	37·88	34·50	34·25 (90)	31·43 (91)
Stafford	36·14	35·42	31·30 (87)	30·04 (85)
Glamorgan	35·96	34·38	34·31 (95)	30·89 (90)
Lancaster	34·48	32·24	28·21 (82)	26·93 (84)
Nottingham	34·44	32·28	30·34 (88)	28·46 (88)
⋮	⋮	⋮	⋮	⋮
Westmorland	27·81	35·32	20·96 (75)	27·02 (77)
Huntingdon	27·46	33·38	23·64 (86)	28·90 (87)
Cornwall	27·09	35·46	21·54 (80)	25·11 (71)
Salop	27·08	34·78	24·71 (91)	31·43 (90)
Hereford	25·44	30·83	22·25 (87)	29·52 (96)

The above illustrations are taken from the upper and lower ends of a table calculated for all the counties. The proportional figures in brackets enable an exact comparison to be made between the decline in the birth-rate as indicated by the crude and corrected rates respectively.

It will be noted that in some instances the differences between the birth-rates of 1881 and 1903 are nearly the same whether the crude or the corrected birth-rate be taken as the test. In other instances, however, the difference is so considerable as to indicate the desirability of eliminating extrinsic arithmetical factors of variation before considering true differences in fertility. Thus in Glamorgan the true reduction is from 100 in 1881 to 90 in 1903, instead of to 95, as indicated by the crude birth-rate; in Cornwall the reduction is from 100 to 71, instead of to 80. These are instances where the real decrease in fertility is greater than that indicated by the crude birth-rates. In other instances the difference is on the opposite side. Thus in Hereford the reduction is from 100 to 96 and not to 87, and in Rutland from 100 to 72 and not to 69. In more than half the counties the changes shown in each individual county between 1881 and 1903 by the corrected were nearly the same as in the crude birth-rates. The correction is not on this account superfluous, for we have to deal not only with (*a*) comparisons of the same community at different times, but also with (*b*) comparisons between different communities. The differences are much more striking when the second set of comparisons is made, as is especially well shown when the corrected birth-rates of the counties of England are compared with those of Scotland and Ireland and with

those of other countries. Even in more limited comparisons it is obviously desirable to eliminate an extrinsic cause of variation which may unexpectedly in any given instance introduce a source of fallacy.

(6) *Counties in order of merit as to birth-rate.*

We use the term Order of Merit as meaning the position in which a county stands in relation to other counties similarly treated, when its actual is stated in proportion to its potential birth-rate. There are two ways of calculating the Order of Merit, both giving the same result. The crude birth-rate of any county may be stated as a percentage of its standard birth-rate; or the corrected birth-rate may be stated as a percentage of the standard birth-rate of England and Wales. Thus if the county of Durham be taken as an example:

Durham, crude birth-rate in 1903 = 34·25,
„ standard „ „ 1901 = 38·04,
„ corrected „ „ 1903 = 31·43,
England and Wales standard „ „ 1901 = 34·91.

Then $\frac{3143}{3491} = \frac{3425}{3804} = \cdot 90.$

The statement of Order of Merit represents the proportion of potentiality to actuality, assuming that the capacities for childbearing are equal in the Swedish and English populations. We know of no reason for doubting that this is so. The counties of England and Wales thus compared show changes in this respect, which if diminution of the birth-rate be regarded, as it is regarded by us, as a matter of serious national import, are of great importance. It is satisfactory to be able to frame such an Order of Merit, from which extrinsic arithmetical considerations, as distinguished from considerations of fertility, have been eliminated.

The figures for 1881 will first be considered. In only six was the crude above the standard or potential birth-rate (= 100). In Rutland it was 104·2, in Cumberland 103·1, in Cornwall 101·6, in Stafford 101·5, in Westmorland 101·2, and in Oxford 100·4. At the other end of the scale in Worcester it was 87·2, in Hereford 88·3, in London 88·6, in Yorks 89·8, in Hants 91·5. Even in 1881 therefore there were in operation causes which were lowering the actual below the potential birth-rate to a very varying extent in the different counties of England and Wales.

In 1901 a very different story has to be told. Not one county is up to the Standard of Merit constituted by Sweden (=100). The highest is Monmouth 94·9, next Durham and Salop 90·0, then Cumberland 89·2 and Glamorgan 88·5. At the other end of the scale are Sussex 69·2, Devon 69·6, Northampton 70·5, Bedford and Cornwall 71·9, and Hants 72·0.

Having compared counties between themselves, we may next consider the changes in each county between 1881 and 1903. The corrected birth-rate of each county is stated as 100, and the corrected birth-rate in 1903 in proportion to this. Thus stated, Monmouth is at the head of the list with 98 in 1903, as compared with 100 in 1881. Hereford comes next, 96, then Durham 91, Salop, Glamorgan, and Worcester 90, Nottingham 88, and Northumberland and Huntingdon 87. At the other end of the scale are Cornwall 71, Rutland 72, Northampton 73, Devonshire and Sussex 74.

Certain counties stand out prominently in order of infertility. Thus Sussex is lowest in the list in 1903, with only 69·2 per cent. of the births it ought to have according to the standard taken. It also gives with three exceptions the heaviest decline in its order of merit. But in 1881 it was already low down on the list, being 33rd on the list of 41 counties, beginning with the county having the highest corrected birth-rate; and it has since 1881 managed to make one of the heaviest percentage decreases from its former low position, and stands in 1903 at the bottom of the list.

The instance of Cornwall is only a little less striking. It has fallen from the third place in 1881 to the 37th place in 1903. Possibly the absence of husbands in South African or other mines, or their return debilitated by lung diseases, may partially account for this instance, which in this way may be exceptional. The other Celtic populations of Wales, the Highlands, and Ireland have all high corrected birth-rates, some of them much higher than any in England.

Rutland shows the second greatest decrease. It has fallen from the 1st to the 32nd place. This result in a purely rural county is remarkable. Devon and Westmorland are however not far behind, and it would appear that the decline in the corrected birth-rate has affected the rural as much as if not more than the manufacturing and mining counties.

REPORT OF AN OUTBREAK OF PLAGUE IN QUEENSLAND DURING THE FIRST SIX MONTHS OF 1904.

By C. C. BAXTER-TYRIE, M.D., C.M., Edin.,
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Plague was introduced into Queensland from Sydney in 1900, and every subsequent year has been marked by an outbreak in Brisbane. In both towns the authorities are unsparing in their efforts to eradicate the factors contributing to its maintenance, but in both places the infection of the rats and their haunts is so extensive that the disease may now be looked upon as endemic in the two cities. The probabilities are, however, that the pest will be harder to eradicate in Sydney, where the buildings are substantially built, and not as in Brisbane light structures of wood which more easily lend themselves to treatment and are comparatively temporary in their character.

During the period stated twenty-nine cases were reported in the State. Twenty-four cases occurred in Brisbane and the immediate vicinity, one at Ipswich, two at Cairns, and two in Maryborough. (43 cases were reported as suspicious and negatived on clinical and bacteriological examination.)

The routine method of notification and report instituted by the Commissioner of Public Health (Dr Ham) is comprehensive and accurate, and reduces the chances of diagnostic error to a minimum.

Immediately on receipt of notification of a suspect, generally by a medical practitioner, the Government Health Officer visits the case, examines it clinically, and takes specimens from the affected gland, blood, or sputum, as circumstances may indicate. Hypodermic syringes and needles in sterilized test-tubes are carried ready for use. Smear preparations are made and part of the material is forwarded to the Government Bacteriologist, who also examines smears and inoculates a serum tube and guinea-pig. The results of the clinical and immediate bacteriological examination are embodied in the Report, to which the subsequent reports on culture and inoculation are appended.

General features of the outbreak.

The progress of the disease and the incidence in the several months are included in the appended table. The figures for the years 1900, 1901, 1902, and 1903 are submitted for comparison.

Month	1900	1901	1902	1903	1904
January			1		
February		1	14	6	7
March		7	20	4	
April	3	7	28	6	7
May	10	12	18	4	12
June	10	3			3
July	14	3			
August	4	1	1	1	
September	5			1	
October	4	1			
November	3				
December	3	1			
Totals	56	36	82	21	29

The majority of the cases, as shown in Appendix B, were associated with the handling and distribution of produce, and it is instructive to compare Appendix B, relating to the occupations of the patients, with Appendix C dealing with the incidence of plague rats on the premises of the various trades. (See p. 328.)

Most of the patients were men in adult life and there were only two females, one of whom was employed in the produce trade. This disparity in the sexes is accounted for by the fact that women are hardly ever employed in the trades from which the cases are derived.

Causes of recrudescence of plague in Queensland.

The last case of plague which occurred in Brisbane in 1903 was on September 11th: the last infected rat was found during 1903 on September 28th. The first infected rat of 1904 was found on January 8th, and the first case of human plague on February 9th.

The factors contributing to and causal of the present outbreak may be considered under importation, rats, seasonal influence, infection by food, and infection by insects.

Importation.

While there is no evidence to adduce in support of the present outbreak of plague being other than purely sporadic, it is well to direct attention to a possible source of infection in the jute bags which are imported from Bombay and Calcutta. The regulations in force prohibit the importation of used produce sacks, and when it is realised that many of the new bags imported from India are made from material served out by the factories to natives who make it up in their own hovels where plague infection is ripe, the necessity will be apparent for submitting these bags to thorough disinfection before allowing their distribution.

Rats.

During the period January 1st to June 30th, 37,254 rats and mice were destroyed; of these 14,755 were examined. 310 rats out of 11,479 were found to be infected, and 3 mice were infected out of 3,276 examined.

The following table illustrating the number of rodents examined for the last three years shows the work done by the Department of Public Health in destruction and examination.

Until the present year mice were included under rats and no distinction was made.

1902

"Rats" examined 1,315
"Rats" infected 96
Percentage of "rats" infected 7·3

1903

"Rats" examined 6,500
"Rats" infected 80
Percentage of "rats" infected 1·23

1904

Rats examined 11,479
Rats infected 310
Percentage of rats infected 2·7
Mice examined 3,276
Mice infected 3
Percentage of mice infected ·90

Totals

Rodents examined 14,755
Rodents infected 313
Percentage of rodents infected 2·1

As will be seen from Appendix D, 78 of the 313 infected rats were obtained from the "Infected Area," and 88 were found during the cleansing operations in one store in South Brisbane. Appendix D also shows the localities from which the rats were obtained during the several weeks of the period under observation. Appendix C sets forth the

incidence of infected rats on the various business premises. It will be noted that the produce premises are responsible for 111 out of a total of 313.

In view of the heavy incidence of plague-infected rats and plague cases associated with the storing of produce a systematic inspection was made of all the metropolitan stores, and cleansing operations instituted. The results of the investigations are embodied in Appendix E. They may be of use to others who may have to deal with similar infective foci.

Wherever an infected rat is found the immediate and neighbouring premises are thoroughly overhauled and search made for other rats, and all sanitary and other defects made good.

Species of infected rats:

Mus decumanus	249
Mus alexandrinus	31
Mus rattus...	27
Species not given	3
Mice	3
Total	313

It is essential that the operations for rat destruction on any premises be conducted continuously and, if possible, completed in one day. If uncompleted the rats invariably migrate during the night. *Mus alexandrinus* is the most difficult to deal with. It rarely burrows, climbs well, seldom remains long in one place, and decamps at the first sign of operations.

Endemic maintenance and dissemination of the disease by rats are the main causes of the annual outbreak of plague in this State. Many other animals are more or less susceptible to plague. Poultry merit particular attention, as not only are they themselves susceptible but their food is a constant attraction to rats. Here until recently it was a common occurrence to find the ground under the cottage houses barred off as a fowl yard[1].

Simpson (Hongkong Report, 1903) puts the case very clearly:—

"The mortality of rats preceding and coinciding with the epidemic is significant and important. Were rats and men attacked concurrently at the beginning of an epidemic the spread of the infection would have to be looked for in some common media containing the infectious material and which were likely to affect both simultaneously. The antecedence of rat to human plague in this and every other

[1] Houses are built on piles to protect them from the ravages of white ants.

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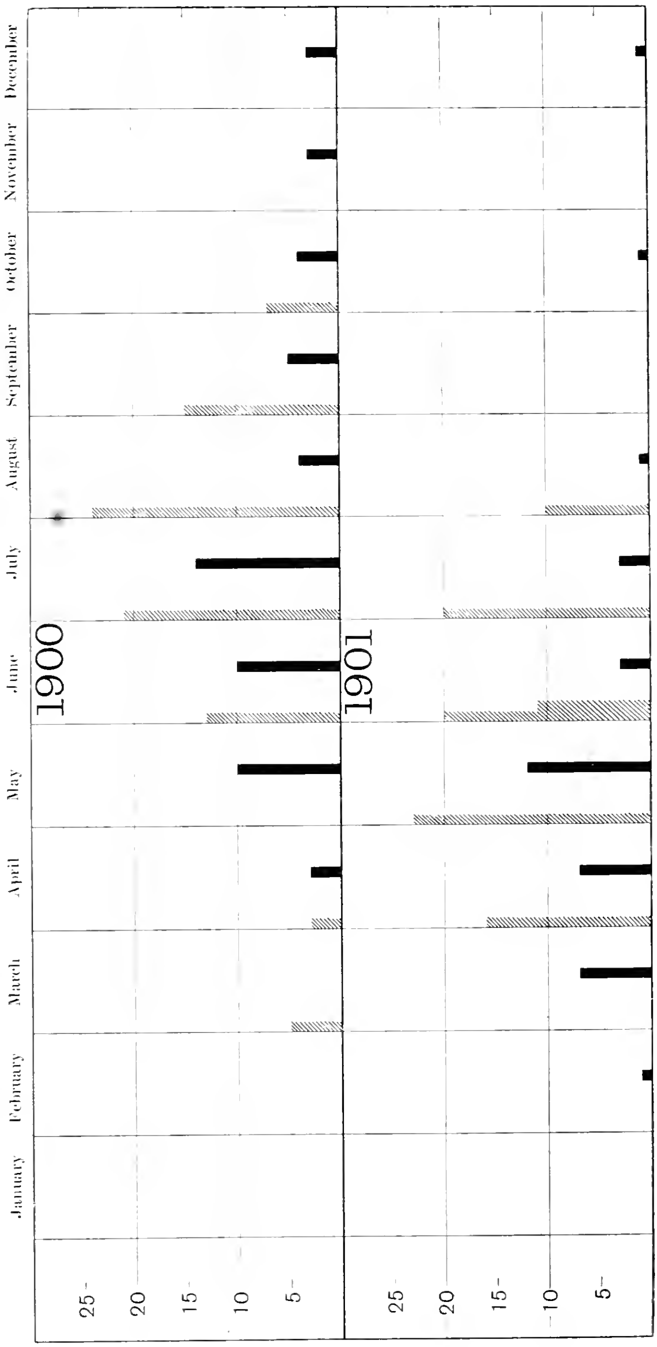
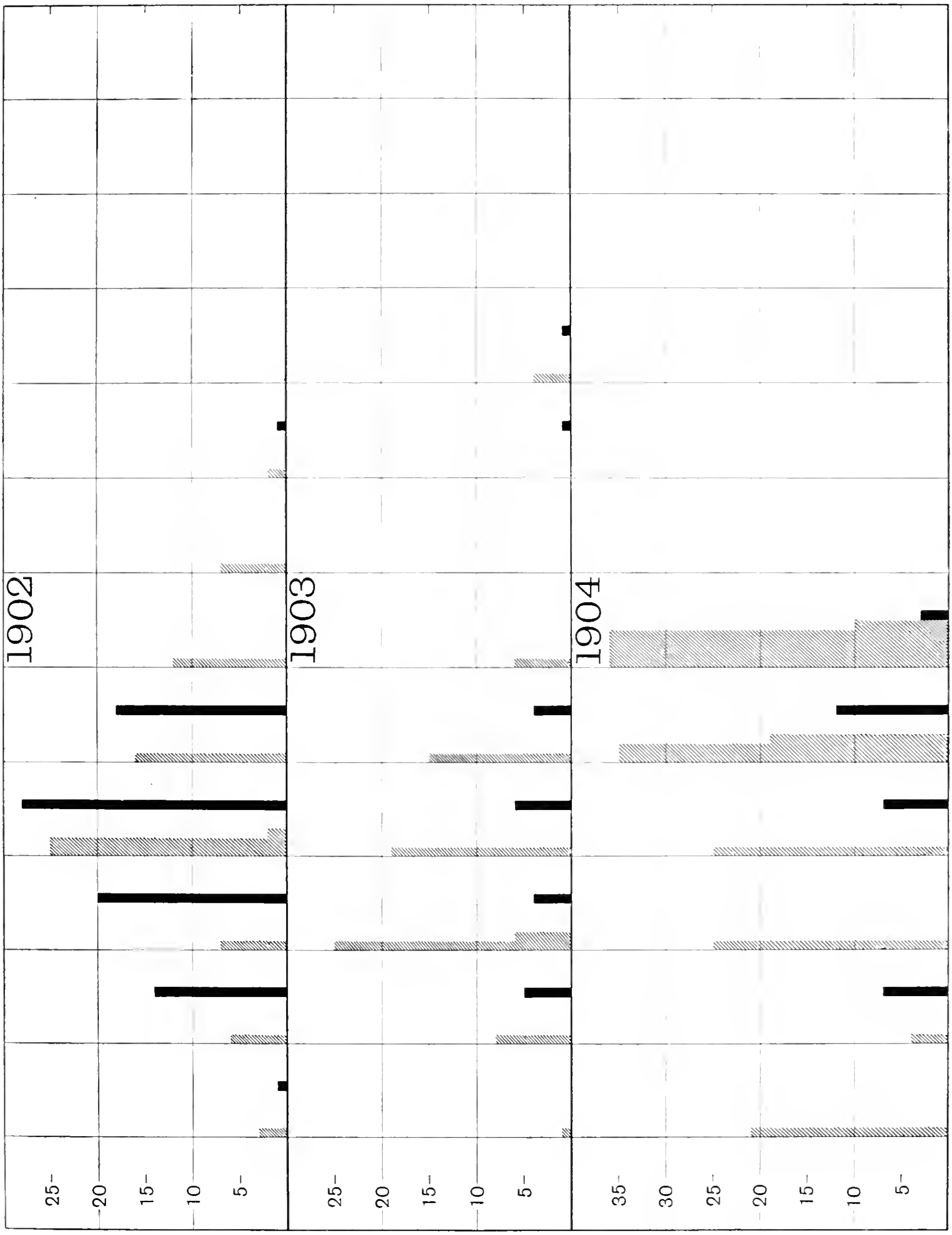
GRAPHIC CHART OF RAT AND HUMAN PLAGUE IN THE YEARS 1900—1901.

Incidence of Rat Plague shewn shaded. *Incidence of Human Plague shewn in black.*




1902
1903
1904
25
20
15
10
5
35
30

epidemic points to a channel of infection which implicates the rats alone primarily. The dissemination of the infection by the rat is necessary to bring it into those channels by which man can become infected."

It is probable that under certain natural circumstances a reduction in the virulence of the organism is effected and a comparative immunity is conferred on the rats. The infection of immigrant rats is, however, severe and their arrival is heralded by a heavy mortality. In the same manner an infected rat imported into a fresh locality produces a similar result. This attenuation of virulence is responsible for the condition known as chronic rat plague. Kolle and Martini[1] first directed attention to its occurrence. Observations and experimental investigations conducted in the Bacteriological Laboratory here by Mr Pound, and discussed by Dr Ham in a State Health Paper, place its existence in Brisbane beyond doubt.

In view of the high probability that infection from rat to rat is mainly by the agency of food it is significant to note that guinea-pigs are easily infected through the digestive tract, in which case there is a special tendency to chronic forms (nodules in various organs including the lungs)[2].

These facts are a clear explanation of the many instances of prolongation of large epidemics and of reappearances of plague after opportunity for infection has apparently disappeared.

It is significant that the rat plague reappeared this year first in the "Infected Area" particularly in connection with the produce stores, and that all the cases with the exception of eight were connected with the produce industry directly or indirectly.

The view that rat to rat infection is effected by insect agency must be greatly discounted in the light of recent investigations into the infectivity of the secretions and excretions of plague-stricken animals. The plague bacillus is found in the mucous secretions, faeces and urine of from 20 to 30 % (see Simpson's Hongkong Report, 1903) of infected rats and there is considerable ground for belief that contamination of food is the chief mode of rat to rat infection. This dissemination of bacilli by the secretions and dejecta explains the retention of the infection in these rat-ridden stores, most of which were at the beginning of the outbreak overcrowded, structurally defective, inadequately ventilated, badly drained, and insufficiently cleansed.

[1] *Deutsche med. Wochenschr.* Jan. 1902.

[2] Bandi and Balistreri, *Zeitschrift für Hygiene*, 1898, xxviii. p. 261.

Seasonal incidence.

Plague in Queensland as in other affected countries possesses special seasons for its prevalence. It is at its lowest between midwinter and the latter part of summer, and reaches its maximum from February to June. There is a special connection between rainfall and plague. Rainfall during an epidemic is invariably followed by an increase of cases.

This increase is probably due to local conditions. The rain drives the rats from the sewers and secluded underground haunts into buildings and situations frequented by men, who are thus exposed to a more proximate infection.

Infection by food.

Rat to Rat. There is abundant evidence to show that food can and does convey the infection of plague from animal to animal; and considering the high proportion of infected rats which have bacilli in the saliva it is probable that food is the main channel by which the disease is naturally spread among rats.

Rats are continually fighting, and eat their companions that have died from plague. They nibble at food and if infected the bacilli are deposited on the remnant and available for infection of a succeeding feeding rat.

Rat to Man Plague is readily communicated to grey monkeys by food which has been exposed to infected rats, and food is probably the source of infection in man where glandular symptoms are absent or secondary to haemal infection; also probably as will be discussed later in many cases where there is apparently only glandular infection.

Infection by insects.

The experience gained in connection with rat and cleansing gangs points to the conclusion that the relation of fleas to the transmission of the disease has been much over-estimated[1]. The men are being constantly bitten by fleas, especially on the exposed arms. The only cases which have occurred have been due to direct inoculation, one by a splinter, and the other by a rat bite. Isolated examples may

[1] Cf. Kolle and Martini, *Deutsche med. Wochenschr.* Jan. 23rd, 1902, and Indian Plague Commission Reports.

occur where a flea may directly inoculate a victim. Bacilli have been found in fleas as in other insects. The theory that every bubo is indicative of direct inoculation by fleas somewhere in its drainage area is untenable. Their most important rôle is as carriers of infection, the same as bugs, flies, etc. Fleas from their environment of dust and dirt necessarily are more closely associated with plague infection. The significance of this association is evident when it is considered that the plague bacillus, if it does not actually have a saprophytic existence, has not infrequently been isolated from such surroundings[1].

Pound[2] narrates an interesting instance of food infection by cockroaches:

The facts are as follows: In a room specially set apart for keeping all inoculated animals are two large stands with wide shelves on which are placed long lead-lined trays about two inches deep, containing carbolic solution; standing in these trays and surrounded by the carbolic solution are the various strong glass jars in which are kept the experimental animals. These jars are about sixteen inches high and about nine inches in diameter. Each jar contains only one animal, guinea-pig, rat, or mouse, as the case may be, and is covered with a mosquito-proof fine wire-gauze lid. On the occasion referred to, in November, 1902, a healthy guinea-pig that was being kept as a control for certain experiments suddenly became sick and after three days died. Post-mortem and bacteriological examination proved that this guinea-pig had died from a generalised form of plague, but no lesion was found to indicate that it had been infected through the skin. A careful examination revealed the fact that in the zinc binding of the wire cover there were several very young cockroaches. These were promptly destroyed. On examination of the covers of the other adjoining jars more young cockroaches were discovered.

It is quite evident that these cockroaches had become hidden in the zinc lining of the covers when the jars were not in use, and standing on the shelf alone, unprotected by the tray of carbolic solution.

As soon as a jar was occupied by an experimental animal the cockroaches that had been hidden from view in the zinc lining during the daytime, would, after dark, crawl down the inside of the jar and feed on the animal's food. Apparently, before their presence was discovered, some of these cockroaches had fallen from a jar containing a plague-infected animal into the carbolic solution, and then swam either to the jar containing the healthy guinea-pig or to the side of the tray, and then hid away in the cover of an empty jar. In any case it was more than probable that the food had become contaminated with bacilli.

In order to ascertain whether the cockroaches had anything to do with the transmission of plague, a healthy guinea-pig was placed in a sterilised jar covered with the usual wire lid, but whose zinc lining was free from cockroaches. The jar was placed on the shelf, but not on the tray. In the course of a few days young

[1] Kitasato, Hankin, Leuman.

[2] *Queensland State Health Report*, 1903.

cockroaches made their appearance, and, as usual, lived during the daytime in the zinc lining. Eventually the guinea-pig sickened and died of plague.

There is sufficient convincing evidence in this one observation to show the extreme danger which exists when insects like cockroaches can gain access to places where plague-infected animals are kept.

After this experience, the whole of the building and everything such as shelves, benches, jars, etc., were subjected to thorough and repeated disinfection, and all holes and crevices carefully closed. The result is that no cockroaches have been seen since; and although every day during the past eight months numbers of plague and healthy guinea-pigs and rats have been kept in the same jars and standing in the same trays, no symptoms of the disease have appeared in any animal unless specially infected.

Synopsis of twenty-five cases under direct observation.

Case I. Saddler: Infected rats discovered on premises during the preceding week. Initial temperature 103° F.; pulse extremely weak and dicrotic; serum 120 c.c. injected subcutaneously, 2nd day; temperature normal 4th day; right upper femoral gland affected; suppuration delayed and limited.

Case II. Schoolboy: Plague next door last year; infected rats from store immediately adjoining; initial temperature 103° F.; pulse weak and dicrotic. Serum 200 c.c. subcutaneously. Temperature normal 10th day; had serum rash. Left upper femoral gland affected.

Case III. Produce hand: Ill twelve days before coming under observation. Initial temperature 100° F.; dicrotic pulse. Temperature normal 7th day; gland resolved without suppuration. No serum was given. Left upper femoral gland affected.

Case IV. Produce hand (female): A bag maker. Initial temperature 104° F.; dicrotic weak pulse. Serum 120 c.c. subcutaneously. Temperature normal 18th day: left upper femoral gland affected and sloughed entirely.

Case V. Schoolgirl: from infected area. Initial temperature 105° F.; pulse 180, weak and dicrotic. Serum 240 c.c. subcutaneously, 120 c.c. intravenously. Temperature normal 17th day; upper left femoral gland affected. Very severe case.

Case VI. Produce-bag merchant: Initial temperature 105° F. Serum 240 c.c. subcutaneously. Temperature normal 5th day. Left upper femoral gland affected.

Case VII. Produce hand: Initial temperature 104° F. Serum 240 c.c. subcutaneously. Left upper femoral gland affected; little suppuration. Temperature normal 4th day. 17 days in hospital, the shortest period of detention of the cases.

Case VIII. Produce hand: Initial temperature 104° F.; pulse extremely weak. Serum 200 c.c. subcutaneously. Died on the second day. Complete renal suppression from time of admission. History of previous renal trouble. Autopsy findings: chronic interstitial nephritis and general infection of the organs with the bacilli. Cultures of *B. pestis* were obtained from all the thoracic and abdominal viscera.

Case IX. Stable hand: Initial temperature 104·6° F. Obtained horse feed from infected store. Serum 200 c.c. subcutaneously; temperature normal 5th day. Left upper femoral gland affected; was not removed to Colmslie.

Case X. Produce hand: Initial temperature 99·2° F. Serum injected subcutaneously. Died on the second day. General blood infection, bacilli were mixed in type but mainly coccal.

Case XI. Grocer and Produce hand (Chinaman): Initial temperature 104° F. Died immediately after being seen, had been ill 4 days. Large bubo implicating the whole left femoral group.

Case XII. Produce hand: Initial temperature 103·6° F.; pulse weak and dicrotic. Serum 102 c.c. subcutaneously. Temperature normal 5th day. Left upper femoral gland affected and resolved without suppuration.

Case XIII. Grocer and Produce hand: Initial temperature 104·6° F.; pulse 120, weak and dicrotic. Serum 320 c.c. subcutaneously. Left upper femoral gland affected; serum rash. Temperature normal 20th day. Gland sloughed *en masse.* Very severe case.

Case XIV. Race-horse trainer: Produce obtained from infected store. Rats dying in numbers prior to his seizure. Initial temperature 105° F. Pulse dicrotic. Serum 240 c.c. subcutaneously, right upper femoral gland affected. Temperature normal 12th day.

Case XV. Oyster-saloon cook: Infected rats from the shop this year. Four days ill before seen; initial temperature 105·4° F. Pulse 152, dicrotic. Serum 120 c.c. subcutaneously. Died shortly after being seen. General blood infection.

Case XVI. Produce hand: Initial temperature 120·6° F., pulse weak and dicrotic. Serum 120 c.c. subcutaneously. Temperature normal 6th day. Right upper femoral gland was affected and resolved without suppuration.

Case XVII. Produce hand: Initial temperature 104·2° F., pulse 90, weak and dicrotic. Serum 120 c.c. subcutaneously. Temperature normal 8th day. Lower left femoral gland affected; sloughed *en masse.* Detained in hospital 46 days. The longest case. There was a sore on the inner side of the left calf which yielded plague bacilli on examination. There was marked lymphangitis between the sore and the saphenous gland. The sore (circular, ¾-inch in diameter) consisted of a central black eschar round which was a ring of purulent vesicles. Surrounding this again was an area of livid, brawny induration. From the same store as in *Case XVI.*

Case XVIII. Sanitary wharf-man: Ill three days; died before arrival of Health Officer. Left upper femoral gland affected. Autopsy: general blood infection and secondary specific pneumonia.

Case XIX. Estate agent: Initial temperature 104·8° F., pulse 104, very weak and dicrotic; left upper femoral gland affected. Serum 240 c.c. subcutaneously. Temperature normal 7th day. Dead infected rat found in offices.

Case XX. Stableman: Initial temperature 102·6° F., pulse 100, weak and dicrotic. Serum 240 c.c. subcutaneously. Left upper femoral gland affected; temperature normal 5th day. Serum rash. Produce from infected store.

Case XXI. Schoolboy: Initial temperature 105° F.; pulse 136, weak and dicrotic. Serum 240 c.c. subcutaneously. Right upper femoral gland affected; temperature normal 6th day. Infected rats at store close by, which he was in the habit of frequenting. Family cat also brought rats to the house from this store.

Case XXII. Produce Store hand: Initial temperature 103·8° F., pulse 104, weak and dicrotic. Serum 480 c.c. subcutaneously. Left inferior external axillary glands

affected, also left femoral. Developed intense blood infection and secondary specific pneumonia and died on the 10th day. The only case where subcutaneous haemorrhages were present; these appeared on the 6th day.

Case XXIII. Draper: Infected rats obtained in the vicinity of the home. Initial temperature 102·8° F.; pulse 96, weak and dicrotic. Serum 120 c.c. subcutaneously. Temperature normal 9th day. Left upper femoral gland affected.

Case XXIV. Baker: Infected rats from bakehouse. Serum 120 c.c. subcutaneously. Died 5th day. General haemal infection. Developed meteorism the second day, and lapsed into an adynamic condition which continued until death.

Case XXV. Student: A remarkable case; arrived in Brisbane a week previous to his attack. When seen by Drs Bancroft and Love was found to have a condition similar to malignant pustule on the nape of his neck, a central eschar within a zone of confluent pustules and surrounded by an area of hard brawny indurations (cf. *Case XVII*). On bacteriological examination plague bacilli were found in large numbers. The lesion was energetically treated by incision and pure carbolic acid. Serum 240 c.c. was administered subcutaneously. The bacilli were evidently all eradicated in the local lesion as there was no secondary adenitis. Initial temperature 104° F.; pulse 112, dicrotic; rapid constitutional recovery. Temperature normal 3rd day. There were no bacilli obtained in the scar after treatment. The scar healed in a month.

Points of clinical interest.

The average stay of the patients in the hospital was 29 days as compared with 50 days in 1903.

The average time that the patients were ill before serum was administered was 2·6 days as compared with 4·16 days in 1903. The reduction of the period as a result of prompt notification of the cases by the profession is a matter for congratulation, as I think to it is due the very substantial reduction in the death-rate shown by the following tables:—

Gross Case Mortality (Fatality).

1903	1904
53·8 %	31 %

Corrected Case Mortality for White Races.

1903	1904
42·7 %	20 %

Prompt notification is imperative to ensure the early administration of serum.

Serum was administered to almost every one of the 43 suspects. The experience is that if the case proves eventually not to be plague no evil results ensue from the injection.

In addition to the usual concomitant constitutional symptoms special attention was directed to the occurrence of albumen in the urine. Albuminuria was found to be present in 80 % of the cases. Its amount of albumen varied from a mere trace to 5 "Esbach" in a fatal case where the immediate cause of death was suppression. Albuminuria was more marked in fatal cases. In the cases in which it occurred it appeared generally about the third day and persisted usually for about a week. Hyaline, granular, and epithelial casts were frequently observed. In one instance blood casts were present and in two blood was present in small amount in the urine. Difficulty in micturition and associated pain were a marked feature at an early stage of the disease in a large proportion of adult males.

In the State Health Report, 1903, Dr Ham emphasises the early advent of a peculiarly soft and dicrotic pulse in plague. The clinical symptoms in the several cases constituting the present epidemic have varied considerably. Some cases have been associated with high temperature: in others there has been little rise of body heat. In some vomiting has been the salient feature; in many diarrhoea; in others both. The pulse rate varied in the initial stages from 70 to 150. But in all (Case XXV excepted) there have been two constant clinical features, viz. adenitis and marked dicrotism of the pulse. The latter is undoubtedly the most constant clinical symptom in plague. Its occurrence in association with femoral adenitis may be regarded as pathognomic of the disease.

Anderson (*Australasian Medical Gazette*, 1904) states that if the bubo be inguinal or femoral there is always swelling and thickening in the corresponding iliac fossa, coupled with tenderness on firm pressure. He regards the sign as peculiar to bubonic plague. I have directed attention to this condition, and although in a few cases I have detected a slight fulness probably due to propagated adenitis, I am unable to endorse his view of the value of the symptom. The tenderness I have found has been no more than one would expect on firm pressure in the immediate vicinity of any acute inflammatory lesion.

Heart-failure is a grave possibility in the early pyrexial stage of the disease; but as soon as the temperature falls the danger of such a catastrophe disappears. Myocarditis is frequently spoken of as the cause of the feeble heart action in plague, but I think that were myocarditis present in sufficient degree to be alone responsible for the state of the heart the eventual rapid and complete restoration of the heart to a normal condition would be impossible. The circumstances point

rather to a toxaemia affecting the vagal centres and the intrinsic cardiac ganglia.

In bubonic plague sufficient attention has not, I think, been accorded to the significance and influence of kidney complications. They are most important, and there is a fertile field for valuable research in this direction to ascertain how much of the albuminuria is due to specific kidney affection and how much to serum injection; and whether serum injection is contra-indicated where there is evidence of renal complications or indicated irrespective of the state of the kidneys.

Diagnosis—clinical and bacteriological.

All the cases were primarily bubonic. Four succumbed to virulent general haemal infection. In two cases death was due to secondary pulmonary infection. There was no case of primary pulmonic plague. In two cases there were primary plague sores from which plague bacilli were obtained. These cases are reported and commented on in the synopses of case records submitted.

The gland initiating the bubo was, with two exceptions, either the upper external or upper internal femoral, and in 19 out of 25 cases the left side was affected, a proportion of 76 %.

This constant involvement of the upper femoral glands cannot be a mere coincidence. It cannot be explained by their relation to the areas drained by them primarily, which is insignificant. They are really secondary glands interpolated on the collecting trunks from the lower femoral glands which receive the lymphatics draining the major portion of the cutaneous surface of the lower limb. In the case where there was a primary sore on the inner side of the calf in the drainage area of the internal saphenous lymphatics, the gland affected was the lower saphenous femoral gland (cf. Case XV).

In a previous section of the report I have directed attention to researches which tend to minimise the hitherto predominant position occupied by the flea hypothesis of infection. These facts relative to the particular glands and their relation to cutaneous drainage go a long way to further discount the hypothesis.

The extreme range in the degree of constitutional symptoms associated with the local adenitis rendered an accurate diagnosis in many cases extremely difficult. One had to obviate the possibility of a simple adenitis being diagnosed as plague, and the patient being removed and exposed to even the very slight possibility of infection in the plague

hospital. On the other hand was the danger of allowing a mild case of plague to escape recognition. The danger in such an event would be from some of the immediate contacts becoming inoculated by the organisms in the discharge from the gland directly, or through the medium of soiled dressings, etc.

Bacteriological diagnosis is by no means always the infallible test it is supposed to be, especially in mild cases. Interesting points are that the severity of the clinical symptoms is very often in direct proportion to the number of bacilli observed in a smear made from the juice of the affected gland: also that when atypical and coccal forms are present in addition to the ordinary form of the bacillus the prognosis is much graver than in cases where the organisms conform to the regular type.

The triple test—smear, culture, and guinea-pig inoculation—give results in these mild cases which vary as follows:—

	Smear	Culture	Inoculation
A	Positive	Positive	Positive
B	Negative	Positive	Positive
C	Negative	Negative	Positive

(A) If bacteria are recognised in the smear preparations, the culture and inoculation tests invariably yield positive results.

(B) The smear sometimes shows no organisms, yet culture and inoculation are positive.

(C) Smear and culture yield negative results, but the inoculated guinea-pig dies. In these cases the guinea-pig often lives from two to five days longer than the usual time, thus further intensifying the difficulty of establishing an early and accurate diagnosis. Indeed in not a few cases the patient has recovered and is again in sound health before the death of the guinea-pig.

Cases falling under Class C present a difficult problem as regards diagnosis and the propriety of isolating them. An interesting circumstance in connection with these cases is that the gland generally resolves, and in the isolated instances where suppuration occurs its area of implication is limited and its course short. There are authorities who assert the existence of a disease called "pestis minor which is not plague." I have no doubt the majority of cases designated as such belong to the category under discussion, and are simply examples of mild plague resultant on an infection by *B. pestis* of low virulence, whose capacity for multiplication is soon overcome by the bacteriolytic power of the body cells and fluids. The local disturbance is therefore

slight and the clinical symptoms comparatively trivial and evanescent. These details support the dictum that *B. pestis* like many other organisms varies in virulence.

The virulence of the bacillus can be increased or diminished artificially *in vitro* and *in vivo* by methods which are well known. Under natural conditions there are doubtless many circumstances which influence and determine the degree of virulence in particular individuals and epidemics.

This conclusion is supported by experimental infection, and the cutaneous method devised by Weichselbaum, and elaborated by Albrecht and Ghon, is invaluable in determining the degree of virulency of the bacilli in any given case. Guinea-pigs and rabbits though excellent for ordinary diagnostic purposes are very unsuitable for testing the degree of virulence of plague organisms.

The lung tissue (cf. Klein, Local Government Board Reports) has a special power of exalting the virulence of *B. pestis*, since guinea-pigs inoculated with organisms from the lung of an animal dying from plague succumb much sooner than others inoculated from the spleen or other organ. Further, the cultural descendants of bacilli taken from the lung retain exalted virulence for several generations. This emphasises the necessity of faithfully observing the usual special precautions adopted in treating pneumonic plague.

With a view of ascertaining the value of the recent discovery of Bell, of Hongkong, that *B. pestis* is present in the blood of every case of plague, and not as hitherto regarded only in septicaemic cases or as an immediate antecedent of death, I have examined smears from the peripheral blood of the last eleven patients admitted to the hospital; I have found plague organisms present in the blood of eight. Three of these cases subsequently died, two from secondary septicaemic plague and one from secondary pulmonary infection, therefore the results obtained from them are not admissible.

The presence of bacilli in the peripheral blood in the remaining cases, however, lends striking support to the view that there is in every case of plague a dissemination of bacilli through the general circulation. Of the three cases in which I failed to detect the bacillus, two were extremely mild and the third showed a purely cutaneous lesion which was treated in time to prevent even glandular infection. This accords with my previously expressed views that the severity of the disease, given the same degree of virulence, is directly proportionate to the number of organisms present. How soon the organisms appear in the

blood I am unable to say until further investigation has been made. I have been unable to detect their presence after the defervescence of the initial pyrexia.

This demonstration of the presence of bacilli as a common occurrence in the general circulation of individuals suffering from plague introduces a further tangible basis for doubting whether buboes are always indicative of local inoculation[1].

Treatment.

The immediate and remote results attending the administration of Yersin's serum were very gratifying. If a *potent* serum is given early and in sufficient dosage nearly every case of bubonic plague in Europeans ought to get well, provided there are no complications such as kidney mischief, etc.

The published experience of clinicians who have used this serum attribute to it very varying values. Some belaud it, others condemn it as useless. The explanation lies in the varying potency of the serum that has been employed. It deteriorates with age, some specimens more rapidly than others. In other instances it will be found that fresh consignments of serum obtained from the Institutes vary in potency. Why this should be so I am unable to explain; possibly it may be due to the idiosyncrasy of the horses used. In all cases, however, it is imperative whenever a fresh supply is used, and from time to time with the stock in hand, to conduct a series of experiments on guinea-pigs or other animals to determine the degree of potency. By this means one is enabled to discard or reject unsatisfactory serums.

The routine treatment is to give 120 c.c. immediately the case is brought under observation. The dose is repeated in twelve hours if the temperature remains up. Rarely is it necessary to give a third dose.

Cold bathing and sponging are invaluable in combating delirium, pyrexia, and insomnia. Antipyrine and its congeners are useless and dangerous.

It is extremely important that a close watch be kept on the

[1] *Note:* My method of making the smear was (all precautions were taken to eliminate possible contamination) to obtain very thin smears from the patient's blood by Cole's method. In a private communication from Dr Bell in which he furnishes further results he informs me that he works with very thick smears which he decolorises. It is a matter of gratification that we have obtained similar results with diverse methods.

performance of the kidney functions until the initial pyrexia has defervesced. Where possible, the urine ought to be tested for albumen every time passed. Sudden and extensive structural damage or functional disturbances as evidenced by a large amount of albumen in the urine may occur any time and demand prompt and vigorous treatment. Urotropin is useful, whilst subcutaneous and intravenous injections of saline are invaluable, and in many cases will satisfactorily tide over a very ominous and grave crisis.

By far the best hypnotic in plague is morphia given hypodermically. A single sufficient dose even up to half a grain is much more efficacious and safer than repetition of smaller doses. The digestive system stands the strain of plague well, and a generous diet may be exhibited with advantage much earlier than is usual in similar febrile diseases.

The successful management of buboes is an important feature in abbreviating the stay of the patient in the hospital. My experience is that premature incision of buboes ensures protracted, intractable, and defective sinuses. If the buboes are allowed to thoroughly mature the plague bacilli are killed by the pyogenic organisms, and when the abscess is opened the whole of the necrosed gland is speedily discharged and specific organisms have either disappeared or do so in a day or two. A clean, healthy cavity is left, the walls of which speedily granulate up.

Sequelae have been rare and insignificant in this epidemic, and, as after typhus, many patients aver that they enjoy better health than previous to their illness.

In conclusion, I have to acknowledge my extreme indebtedness to the Commissioner of Public Health for his encouragement and kindness in indicating lines of research, and the facilities he has accorded me of access to his investigations and reports.

APPENDIX A.

Brisbane Rodents examined, not examined, infected, and destroyed, for 6 months ending 30th June, 1904.

Date week ending 1904	Rats examined	Rats infected	Mice examined	Mice infected	Rats not examined	Mice not examined	Total rodents destroyed
January 9	518	1	—	—	15	—	534
,, 16	497	1	—	—	24	—	522
,, 23	507	6	—	—	21	—	534
,, 30	677	13	—	—	17	—	707
February 6	506	—	—	—	42	—	548
,, 13	470	1	—	—	34	—	505
,, 20	394	1	—	—	27	—	422
,, 27	529	—	—	—	462	8	999
March 5	458	4	—	—	1251	560	2273
,, 12	492	3	9	—	1477	1593	3574
,, 19	431	5	38	—	1020	1777	3271
,, 26	584	4	195	—	1202	1975	3961
April 2	358	10	186	—	916	1585	3055
,, 9	358	4	247	—	401	482	1492
,, 16	406	7	249	—	241	684	1587
,, 23	443	3	299	—	231	739	1715
,, 30	497	11	345	—	140	417	1410
May 7	428	15	237	—	215	221	1116
,, 14	379	12	222	—	345	331	1289
,, 21	340	15	244	2	263	328	1192
,, 28	300	27	121	—	224	224	896
June 4	364	80	162	1	289	244	1140
,, 11	430	28	129	—	297	250	1134
,, 18	547	36	221	—	277	277	1358
,, 25	371	9	208	—	251	279	1118
,, 30	195	14	163	—	178	352	902
Totals	11479	310	3276	3	9860	12326	37254

Percentage of rats infected 2·7. *Bonus paid £399. 0*s*. 11*d*.
,, ,, mice infected ·09. Wages paid £1183. 15*s*. 2*d*.
£1582. 16*s*. 1*d*.

* Bonus 6*d*. per rat, 3*d*. per mouse.

APPENDIX B.

Occupations of Patients who suffered from Plague during 1904.

Draper	1
Estate Agent	1
Grocers	2
Produce Store employees	9
Saddler	1
Oyster Saloon Cook	1
Sanitary Wharf labourer	1
School children	3
Storeman	1
Student	1
Stablemen	3
Total	24

APPENDIX C.

Number of Infected Rats, etc., found in the various Business Premises in the Metropolitan Area for six months ending 30th June, 1904.

Advertising Agent	1
Arcade	1
Bacon Store	1
Bag and Bottle Store	1
Baker	1
Blacksmiths and Ironmonger	5
Boarding Houses	3
Bootmakers	2
Butchers	12
Butter Factory	1
Cafés	11
Carpenter	1
Chinese Dealers	4
Coachbuilder	4
Confectioner	2
Courier Buildings	3
Dentist	1
Draper	1
Drill Shed	1
Eating, Fruit, and Oyster Saloons	6
Farmer	5
Fish dealer	2
Government Buildings	2
Brought forward	71
Grocers	20
Hardware and Fancy	1
Hotels	15
Municipal Baths	3
Municipal Markets	12
Music Shop	1
Printers	2
Produce Stores	111
Railway Premises	2
Residences	33
Saddler	4
School	1
Secondhand Stores	2
Shipping Office	1
Stationers	2
Undertaker	1
Unknown	2
Upholsterer	1
Wharves and River-banks	20
Wholesale Warehouses	7
Wood Depot	1
Total infected Rodents	313

Of the above number 3 were mice, the remainder rats.

APPENDIX D.

Brisbane Analysis of Plague-infected Rats, etc., for 6 months ending 30th June, 1904.

1904 Week ending	City	Kangaroo Point	Valley	New Farm	Spring-hill	Petrie Terrace	New-stead	Bowen Hills	Infected Area	South Brisbane	Woolloon-gabba	West End	River & Wharves	Outside Authorities	Totals
Jan. 8	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
,, 15	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
,, 22	—	—	—	—	—	—	—	—	4	—	—	—	1	—	5
,, 29	5	—	—	—	—	—	—	—	7	—	—	—	2	—	14
Feb. 5	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
,, 12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
,, 19	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
,, 26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
March 4	—	—	—	—	—	—	—	—	4	—	—	—	—	—	4
,, 11	—	—	—	—	—	—	—	—	3	—	—	—	—	—	3
,, 18	—	—	—	—	—	—	—	—	3	—	—	—	1	—	4
,, 25	—	—	—	—	—	—	—	—	4	—	—	—	—	—	4
April 1	2	—	1	—	—	—	—	—	7	—	—	—	1	—	11
,, 8	3	—	—	—	—	—	—	—	1	—	—	—	—	—	4
,, 15	2	—	—	—	—	1	—	—	3	—	—	—	1	—	7
,, 22	—	—	—	—	—	—	—	—	2	—	—	—	—	—	2
,, 29	4	—	—	—	—	—	—	—	4	1	—	—	2	—	11
May 6	3	—	2	—	1	—	—	—	5	1	—	—	—	—	12
,, 13	5	—	3	—	2	—	—	—	4	1	—	—	—	—	15
,, 20	3	—	1	—	—	4	—	—	5	4	—	1	—	—	18
,, 27	4	—	—	—	1	—	—	—	2	1	6	—	1	1	16
June 3	4	—	2	—	—	—	—	—	3	4	73	3	1	—	90
,, 10	4	—	—	—	—	1	—	—	1	1	7	1	6	—	21
,, 17	11	—	3	—	1	9	—	—	9	1	1	3	—	5	43
,, 24	1	—	—	—	—	—	—	—	1	3	—	—	1	—	6
,, 30	2	—	1	—	—	8	—	—	3	—	1	—	4	—	19
Total	54	0	13	0	5	23	0	0	78	17	88	8	21	6	313

APPENDIX E.

THE CONDITION OF THE BRISBANE PRODUCE STORES.

The continued incidence of plague-infected rats and plague cases associated with the handling of produce constrained the issuing of instructions by the Commissioner for a systematic inspection and cleansing of the produce stores.

The first conclusion forced upon me was that any building was evidently considered good enough for the storage of grain and fodder. Many of the structures had previously been used for such purposes as omnibus stables, blacksmiths' shops, etc. In most cases where the buildings had been built specially they were of the flimsiest nature, and no attempt had been made to render them rat-proof.

Construction (Brick buildings): Of thirty stores cleansed fifteen were constructed of brick, and where cellars occurred the floors were either concrete or asphalt. Eleven were satisfactory, requiring only such minor improvements as wire netting on windows and ventilation openings, and doors made rat-proof.

The remaining four were unsatisfactory and exhibited such faults as bad drainage, insanitary stabling, earthen floors, and general dilapidation.

Wood and iron buildings (15): One was well ventilated; four in fair condition; the remaining eight were unfit for use as produce stores and could not have been made rat-proof without incurring costs out of proportion to their value. They were therefore condemned.

Incidence of plague rats.

In the thirty stores cleansed and disinfected were found 541 dead rats and 957 live rats; 305 dead mice and 607 live mice. Of the 1492 rats 109 (7·30 %) were infected; and of the 912 mice 2 (·22 %) were found to be infected.

Incidence of plague cases.

Of the 24 persons who suffered from plague within the Metropolitan Area nine (37·5 %) were directly employed in the produce business, and three were engaged in stable duties and in frequent contact with produce.

Storage of produce.

The method adopted for storing by merchants in the Area is very objectionable. The produce is dumped on the floor and then piled up from eight to twelve bags high, thus providing ample cover for rats. The fact frequently referred to by merchants that the stock is being constantly changed and turned over does not remove the objection, as a store is never completely emptied and seldom half emptied, and, as long as any stock is left the rats are able to remain in hiding, and as new stock comes in they again spread themselves out.

In support of this contention it may be stated that the total number of rodents caught in produce stores during the $2\frac{1}{2}$ months that the special cleansing operations were being carried on was $6\frac{1}{2}\,\%$ of the total number caught in the whole Metropolitan Area for six months.

If produce is stored on the floor it is impossible to keep the premises clean, and as chaff falls from burst bags, etc., accumulations form between them which cannot be cleared away until the produce is sent out; and it is seldom done then, but new stock is generally dumped down in the position from which the old stock was removed.

Some of the stores are too small for the business done in them: consequently, overcrowding is common and tends to keep premises dirty.

Recommendations.

That all buildings in which it is intended to store produce should be built of brick and cement or concrete, and that all cellar floors should be of concrete on solid foundation to prevent rats burrowing under same; that all cellar walls should be rendered tight inside with cement mortar. That all doors should fit closely to floors when shut; that all air or light openings should be covered with stout wire netting to prevent the ingress of rats, or that such stores should be built of iron frames sheeted with stout corrugated iron with concrete floors as above, and windows and doors similarly fitted.

That all drains be trapped and ventilated and sufficient surface drainage provided to keep yard in a clean and dry state. That stables be of sufficient size, and that stalls be floored with impervious material and the bedding changed daily. That stall floors have a decided fall outward to a surface drainage through trapped gully.

That manure receptacles of impervious material, with floor somewhat above level of ground and of concrete, should be provided in all cases where there are stables, and that the receptacles be provided with proper cover and thorough means of ventilation, and that "weep holes" be provided into a surface drain. All surface drains should be of concrete or hard burned bricks on edge, properly bedded and grouted in cement mortar.

That all earth closets should have concrete floors and should be kept regularly lime-washed.

That all produce should be stacked on sparred stands at least 12″ off the floor, to enable the floor to be periodically cleansed under stock and that, if possible, the produce be not stacked against walls.

LACTOSE-FERMENTING BACTERIA IN FAECES.

By ALFRED MacCONKEY, M.B., D.P.H.

(Senior Assistant Bacteriologist, Serum Department, Lister Institute of Preventive Medicine.)

The following experiments were begun with the object of ascertaining the distribution in nature of certain lactose-fermenting organisms which are by some grouped under the name *Bacillus coli*, but by others are regarded as belonging to a different class of organisms; a difference of opinion which is most probably the principal factor in causing the value of *B. coli* as an index of pollution to be such a vexed question among bacteriologists. The investigation, however, resolved itself into a search for the *B. lactis aerogenes* principally, and secondarily for the other lactose fermenters. Incidentally certain other points are referred to which it was thought might prove of interest though not actually pertaining to the subject of the paper.

For facility of reference the paper has been divided into sections:—

Section 1. Media—Action of potassium iodide—Methods.

Section 2. Characters of certain lactose-fermenting bacilli; Voges and Proskauer's reaction.

Section 3. Effect of environment upon biological characteristics.

Section 4. Question of preliminary incubation in a liquid medium.

Section 5. Experiments:—

(*a*) Examination of human faeces.

(*b*) Examination of animal faeces.

(*c*) Examination of milk.

(*d*) Examination of animal faeces when the animal was fed on a partially sterilised diet.

(*e*) Examination on successive occasions of a mixture of tap-water and human faeces.

Section 6. The fermentation of starch and inulin.

Section 7. Voges and Proskauer's reaction as a test for the presence of *B. lactis aerogenes* and *B. cloacae*.

Section I.

A. *Media.*

In these experiments the ordinary nutrient media were used as well as bile-salt media[1]. The basis of the latter consisted of a stock solution composed of:—

Sodium taurocholate (commercial),	0·5 gms.
Peptone (Witte),	2·0 gms.
Distilled water,	100 c.c.

For liquid media there is added to this stock solution 0·5 % of a 1 % solution of neutral-red and 0·5 % of glucose, or 1 % of the other carbohydrates or alcohols, as the case may be, and the medium is distributed into Durham's fermentation tubes[2] and sterilised in the steamer for 10 minutes on each of two days, great care being taken not to overheat the medium. If it be thought advisable white of egg may be used to clear the medium.

Bile-salt agar is made by dissolving 1·5 %—2 % agar in the stock solution. This is best done in the autoclave. The medium is cleared with white of egg and filtered. After filtration the same amount of neutral-red is added as in the case of the liquid media. Some workers add alkali to this agar but I much prefer it without, as the increased alkalinity delays the appearance of the acid reaction in the case of some organisms. Neutral-red (suggested in this connection by Grünbaum and Hume, 1902) is used as an indicator in preference to litmus because the medium is light-coloured and the differentiation much sharper. The solution of the dye should be freshly prepared, as with old solutions the result is often not so satisfactory. The addition of glycerine is to be avoided, as it seems to interfere with the sharp differentiation usually obtained. The various carbohydrates and alcohols are added, in the same proportions as for liquid media, any time after filtration, the particular kind or kinds to be added depending upon the purpose for which the agar is to be used. It is convenient to follow the plan of Drigalski and Conradi, and distribute the agar into small flasks con-

[1] *Thompson Yates Laboratories Reports*, 1900—1901.

[2] *Brit. Med. Journ.*, Vol. I. p. 138.

taining about 80 c.c., which is enough for three Petri dishes of the ordinary size. A consideration of the fermentation-reactions of the various organisms shows that by the use of certain substances, either alone or in combination, we can separate organisms by means of colour reactions. Thus, if lactose alone be added to the agar, only the lactose-fractors will produce acid and show pink colonies; if dulcite alone only the dulcite fermenters; if sorbite alone only the sorbite fermenters, and so on. This is the idea underlying all such differential media as Wurtz litmus-lactose agar, or the litmus-lactose-nutrose agar of Drigalski and Conradi. If this principle be extended by combining two or more of these carbohydrates or alcohols in the agar, we can obtain a larger number of organisms giving coloured colonies, and can thus if desirable exclude them, leaving only the colourless colonies for examination. Thus by using lactose and dulcite we include Gaertner's bacillus among the acid producers. The addition of sorbite gives an agar upon which the *Bacillus typhosus* will produce coloured colonies. Cane-sugar will exclude *B. proteus vulgaris*, and mannite one of the varieties of *B. dysenteriae*, that is, of course, on the presumption that only colourless colonies are to be taken for further investigation. It must, however, be clearly understood that it is not suggested that exactly similar results will be obtained always when working with the organisms in unknown mixtures, as in such cases we must take into account the possibility of the presence of transition forms between one group and another, and of bacilli which may have been weakened as regards their fermentative powers by unfavourable environment. Still, making allowance for both these possibilities, we can certainly lighten our labour in searching for, say, *B. dysenteriae* by excluding all those organisms in the mixture which at the time of experiment are capable of fermenting lactose, dulcite, sorbite, and cane-sugar, and if desired mannite also.

On bile-salt neutral-red lactose agar the surface colonies of *B. cloacae*, after about 20 hours in the incubator at 37° C., are usually found raised and opalescent, and some may have a red centre. With lapse of time they tend to run together into masses of mucoid-looking material with spots of deep red in them. The centres of all those colonies which remain discrete become red inside 48 hours. In 3 days the red colour may have entirely disappeared, being succeeded by a brownish-yellow colour. On a bile-salt neutral-red agar containing lactose 1 % and sorbite 0·5 % the *B. enteritidis* (Gaertner), after 24 hours' incubation at 37° C., gives a pink growth, which usually changes to brownish-yellow during the succeeding 24 hours.

With the object of stimulating the growth of *B. typhosus* bile-salt agar has been modified by the addition in varying percentages of the following substances:—Urea, asparagin, Liebig's extract, nutrose, somatose, roborat, plasmon, Nährstoff Heyden, $CaCl_2$, Na_2HPO_4, KNO_3, KI, serum, and a solution of alkaline-haematin. This latter solution was made by dissolving 7 grammes of haemoglobin in 10 c.c. of $\frac{n}{1}$ NaHO, and diluting to 100 c.c. with distilled water. Na_2HPO_4 seemed distinctly deleterious. Of the others only 5 had any apparent stimulating effect. These were:

$CaCl_2$ in the proportion of	0·034	per cent. (cf. Gabritschewsky),
Serum	1·0	,,
Alkali-haematin solution	1·0	,,
KNO_3	0·5	,,
KI	0·5	,,

B. *Action of Potassium Iodide.*

With regard to potassium iodide Elsner (1896) was the first to suggest its use in culture media. He states that his acid potato gelatin with KI 1 % inhibits most organisms except the *coli* and *typhosus* groups, and that even *B. proteus* and *B. mesentericus* do not grow well upon it. L. Grimbert (1896) disputes this statement and says that by adding non-sterilised water to tubes of Elsner's medium he obtained growth of non-pathogenic liquefying bacteria. A. Moore (1902) when using Elsner's medium found that the development of liquefying organisms frequently spoilt his plates, and to avoid this he made use of a potassium iodide potato agar, which, however, was alkaline and not acid as recommended by Elsner. Fermi (1898), tested the effect of a large number of chemical substances upon some 80 odd varieties of organisms. His stock culture medium was a neutral agar. He found *B. typhosus* would not grow in contact with more than 0·3 % potassium iodide; while *B. coli* could not withstand more than 0·75 % of the same salt.

My experiments lead me to conclude that on bile-salt lactose agar with the addition of KI 0·5 %—1 % the *B. typhosus* gives larger surface colonies than on the same agar without KI[1]. It must be remembered that the medium is alkaline to litmus and not acid, like Elsner's gelatin.

[1] The growth of *B. pestis* on bile-salt agar is also stimulated by the addition of 0·5 % KI.

A few experiments showing the effect of KI are given below:—

B. proteus vulgaris, *B. cloacae*, and *B. typhosus* were all grown in tubes of bile-salt mannite broth containing 0·5 % KI and were compared with the same organisms grown in control tubes without KI. No difference in the amount of growth as evidenced by turbidity of fluid and amount of acid or gas production could be made out.

B. typhosus was inoculated into two fermentation tubes of nutrient broth containing 2 % glucose, and to one tube 1 % KI was added and to the other 2 % KI. After 24 hours' incubation at 37° C. there was no apparent difference in the amount of growth in the bulbs, but the fluid in the closed arm of the 2 % KI tube was much clearer than that in the other tube. After 48 hours' incubation no difference could be made out between the two tubes.

Potassium iodide 0·5 % was added to broth containing 2 % glucose, and the medium was distributed into three fermentation tubes which were inoculated respectively with *B. coli communis* (Escherich), *B. capsulatus* (Pfeiffer), and *B. cloacae* (Jordan). After 3 days' incubation at 37° C., the results were:—

	Amount of gas % of closed arm	$\frac{H}{CO_2}$	Voges and Proskauer's reaction[1]
B. coli communis (Escherich)	30	$\frac{3}{2}$	–
B. capsulatus (Pfeiffer)	90	$\frac{1}{2}$	+ after 24 hours' standing.
B. cloacae (Jordan)	100	$\frac{1}{2}$	trace after 24 hours' standing. + after 48 hours' standing.

[1] See below, p. 350, hereinafter referred to as V. and P. reaction in the tables.

Nutrient broth containing 2 % glucose was filled into fermentation tubes, and 1 % KI was added. The tubes were inoculated and incubated at 37° C. for 48 hours, when the results were:—

	Amount of gas % of closed arm	$\frac{H}{CO_2}$	Voges and Proskauer's reaction
B. coli communis (Escherich)	45	$\frac{3}{2}$	–
B. lactis aerogenes (Escherich)	80	$\frac{5}{4}$	+
B. cloacae (Jordan)	100	$\frac{1}{2}$	+
B. proteus vulgaris	10	$\frac{1}{0}$	–

A broth composed of glucose 1 %, sod. taurochol. 0·5 %, peptone 2·0 %, aq. dist. 100 c.c. ± 0·5 % KI was used in a similar way. After 48 hours' incubation at 37° C., the results were:—

	Without KI			With KI 0·5 %		
	Gas amt. % of closed arm	$\frac{H}{CO_2}$	V. and P. reaction	Gas amt. % of closed arm	$\frac{H}{CO_2}$	V. and P. reaction
B. lactis aerogenes (Escherich)	40	$\frac{1}{1}$	+	70	$\frac{2}{3}$	+
B. capsulatus (Pfeiffer)	60	$\frac{2}{3}$	+	60	$\frac{1}{2}$	+
[1]T. W. F. 46	40	$\frac{1}{1}$	+	70	$\frac{2}{5}$	+
B. cloacae (Jordan)	70	$\frac{1}{2}$	+	70	$\frac{2}{5}$	+
B. coli communis (Escherich)	25	$\frac{2}{1}$	–	40	$\frac{2}{1}$	–

[1] This organism was isolated from faeces and will be referred to later in another experiment.

Just previously the same batch of broth had given :—

B. lactis aerogenes	45	$\frac{1}{1}$	+	33	$\frac{2}{1}$	+
B. cloacae	20	$\frac{1}{1}$	+	25	$\frac{1}{1}$	+

The results obtained with a similar broth but containing lactose instead of glucose were :—

	Without KI 24 hours at 37° C.			Without KI 48 hours at 37° C.			With KI 0·5 % 48 hours at 37° C.		
	Gas %	$\frac{H}{CO_2}$	V. and P. reaction	Gas %	$\frac{H}{CO_2}$	V. and P. reaction	Gas %	$\frac{H}{CO_2}$	V. and P. reaction
B. *lactis aerogenes* (Escherich)	20	$\frac{9}{5}$	+	40	$\frac{1}{1}$	+	30	$\frac{1}{2}$	+
B. capsulatus (Pfeiffer)	5	$\frac{1}{0}$	–	20	$\frac{4}{1}$	-	5	$\frac{1}{0}$	–
B. coli communis (Escherich)	10	$\frac{6}{1}$	–	20	$\frac{3}{1}$	--	10	$\frac{6}{1}$	–

In the case of *B. cloacae* the growth in the bulb of the fermentation tube was good, but in the closed arm it was scanty.

In another experiment neutral nutrient bouillon was used instead of 2 % peptone water to make a bile-salt-potassium-iodide-lactose broth. After 48 hours at 37° C.,

B. capsulatus gave 10 % of gas with a ratio of $\frac{5}{3}$ and no V. and P. reaction.
B. lactis aerogenes ,, 90 ,, ,, ,, $\frac{4}{9}$ and a marked V. and P. reaction.
B. cloacae ,, 7 ,, ,, ,, $\frac{1}{0}$ and no V. and P. reaction.
B. coli communis ,, 25 ,, ,, ,, $\frac{4}{1}$,, ,, ,, ,,

Another tube of *B. cloacae* after 7 days' incubation showed :—

$$45\ \%\ \frac{H}{CO_2}=\frac{5}{3}\ \text{V. and P. reaction marked.}$$

A broth of similar composition containing 1 % of cane-sugar as the carbohydrate, instead of glucose or lactose, yielded, after 48 hours' incubation at 37° C., the following results :—

	Without KI			With KI		
	Gas %	$\frac{H}{CO_2}$	V. and P. reaction	Gas %	$\frac{H}{CO_2}$	V. and P. reaction
B. cloacae (Jordan)	100	$\frac{1}{2}$	+	about 5	Tube broken before KHO added.	
B. capsulatus (Pfeiffer)	35	$\frac{1}{1}$	+	50	$\frac{2}{3}$	+
B. lactis aerogenes (Escherich)	30	$\frac{1}{1}$	+	40	$\frac{2}{3}$	+
B. coli communis (Escherich)	–	–	–	–	–	–
B. acidi lactici (Hüppe)	–	–	–	–	–	–
B. pneumoniae (Friedländer)	One bubble	$\frac{1}{0}$	–	One bubble	$\frac{1}{0}$	–
B. neapolitanus (Emmerich)	One bubble	$\frac{1}{0}$	–	One bubble	$\frac{1}{0}$	–
B. cloacae, inoculated from one above	75	$\frac{1}{2}$	+			

and a third trial:—

B. cloacae,						
24 hours	20					
48 hours	...	...	...	7		
48 hours	70					
3 days	90	$\frac{2}{3}$	+			
72 hours	...	...	...	14		
4 days	...	...	...	25		
5 days	...	...	...	50		
6 days	...	...	...	50	$\frac{1}{2}$	+

As Drigalski and Conradi recommend Krystall-Violett as an inhibitor of air organisms it was thought that this dye might on occasion be with advantage added to the broth. A few trials made it evident that this could be done without interfering either with the gas reaction or the production of Voges and Proskauer's reaction.

Taking these results all together it may be said:—

(1) that the addition of KI to bile-salt media may tend to delay the anaerobic growth while not interfering with multiplication aerobically; and

(2) that Voges and Proskauer's reaction is more constant than the "gas-reaction" of Theobald Smith as the former appeared in some cases when the latter was not characteristic.

C. *Methods.*

The method adopted was to emulsify a portion of the sample in a tube of bile-salt broth and to place this tube at 37° C., for, usually, 18—24 hours, but occasionally 48 hours were allowed to elapse before

plates were made. Gelatine plates were poured in the usual way, but agar plates were made after the manner of Drigalski and Conradi, *i.e.*, the melted agar was poured into the plates, allowed to solidify and dried in the warm incubator for an hour or so. A loopful of the broth culture was put on the surface of one of the plates and rubbed over it with a bent glass rod, the same rod (without recharging) being used to inoculate a second and third plate. The plates were incubated at 37° C., for 24—48 hours. Ten colonies were, as a rule, taken from each set of plates and worked through the following media:—nutrient broth, nutrient agar, nutrient gelatine, litmus-milk, and bile-salt broths containing respectively glucose, lactose, mannite, cane-sugar, and dulcite. The cultures were also tested as regards their behaviour towards Gram's method of staining. At first potato was also used and media containing glycerine, starch and inulin, but it was soon found that these media had no great value in these experiments and so their use was discontinued.

Section II.

Characters of certain Lactose-fermenting Bacilli.

As in later sections we shall be concerned with lactose-fermenting bacilli it is necessary that at the outset we should have a clear idea of the biological characters of some of the best known of these organisms, so that a comparison can be made between them and those isolated during the experiments. For this purpose I have collected the descriptions given by certain authors of the following bacilli:—

B. coli, *B. acidi lactici* (Hüppe), *B. pneumoniae* (Friedländer), *B. lactis aerogenes* (Escherich), and *B. cloacae* (Jordan).

Of these we have most knowledge concerning the *B. coli* as it has been the most carefully studied by a large number of workers. The principal characters ascribed to this organism have been gathered together in a table for ease of comparison and any special points are mentioned afterwards. The other organisms do not lend themselves to tabular description and so merely a statement has been given of the opinions expressed concerning them by the various authors mentioned.

Theobald Smith (1895) lays stress upon the amount of gas produced and upon the ratio of H to CO_2 as of value in separating *B. coli* from the *B. lactis aerogenes* and *B. cloacae*. In dextrose broth *B. coli* produces sufficient gas to fill half of the closed arm of the fermentation

TABLE A—SHOWS SOME OF THE REACTIONS ASCRIBED TO *BACILLUS COLI*[1].

	Motility	Glucose	Lactose	Cane-sugar	Milk	Indol	
Theobald Smith (1895)	+	+	+	±	A & C	...	$H : CO_2 :: 2 : 1$.
Grimbert (1896)	+	+	+	−	A & C	+	Dextrin +, mannite +, glycerine −, dulcite −.
Lembke (1896)	+	+	+	...	A & C	+	$\frac{H}{CO_2} = \frac{1+}{1}$.
Ehrenfest (1896)	+	+	+	...	A & C	+	
Orlowski (1897)	+	+	...	...	A & C	+	H_2S +.
Rothberger (1900)	±	+	...	...	A ± C	±	
Radziewsky (1900)	±	+	+	...	A + C	+	
Weissenfeld (1900)	±	+	...	...	A ± C	±	
Durham, H. E. (1900—1901) ...	+	+	+	±	A + C	±	Acts upon various other carbohydrates and alcohols.
Ford, W. W. (1901)	+	+	±	±	A + C	+	
Abbott (1902)	±	+	...	...	A + C	+	Pink colonies on litmus-lactose-agar; rarely A only in milk; growth on potato is always visible.
Muir and Ritchie (1902)	+	+	+	...	A ± C	±	
Hewlett (1902)	+	+	+	...	A + C	+	$\frac{H}{CO_2} = \frac{2}{1}$.
Winslow (1902)	...	+	...	...	A + C	+	Reduction of nitrates; agar growth not visible; reddens litmus-lactose-agar; no liquefaction of gelatine in 7 days.
Irons (1902)	...	+	+	±	A + C	...	$\frac{H}{CO_2} = \frac{1+}{1}$.
Houston, A. C. (1903)	±	+	+	±	A + C	+	
Horrocks (1903)	±	+	+	±	A + C	±	$\frac{H}{CO_2} = \frac{1}{1}$ or $\frac{1+}{1}$; acid in litmus-whey; starch unaffected; gelatine growth never wrinkled.
Jordan (1903)	+	+	+	±	A + C	±	$\frac{H}{CO_2} = \frac{2}{1}$; gelatine non-liq. in 10 days.
Brown, R. T. (1903)	+	+	+	+	A + C	+	Reduces nitrates; H_2S.
Lehmann and Neumann (1904) ...	+	+	+	±	A + C	+	
Johnson (1904)	...	+	...	...	A + C	+	Reddens litmus-lactose-agar; reduces nitrates.
Eyre (1904)	+	+	+	±	A + C	+	Reduces nitrates. Maltose, galactose, mannite, glycerine and dextrine all +.
Savage (1905)	±	+	+	±	A + C	±	

[1] Throughout these experiments the sign +, when used in connection with carbohydrates or alcohols, means that acid and gas was produced. A = acid, C = clot.

tube, the reaction of the medium is very acid, and the ratio of H to CO_2 is as 2 to 1. The acidity of 100 c.c. of bouillon = 5 c.c. of $\frac{n}{1}$ alkali, the indicator being phenol-phthalein.

Grimbert (1896) considers the property of fermenting cane-sugar is not usual among the *coli* group.

Grimbert and Legros (1900) distinguish the *coli* group from the Friedländer group by

(1) its motility,

(2) the absence of capsules in the blood of inoculated animals,

(3) the production of indol in solutions of peptone.

Rothberger (1900) thinks that the motility of this organism varies very much; the same strain being motile at one time and apparently non-motile at another. The stock bacillus of the laboratory in which he worked was a motile bacillus giving the usual growths on agar and gelatine, general turbidity in broth, gas in sugar media, feeble indol, and no clot in milk in 10 days.

Radziewsky (1900) isolated 64 *coli* from faeces by means of gelatine plates, from which he picked off only the typical "vine-leaf" colonies. The resulting cultures were plated and re-plated to ensure pure growths. He found that some of these pure cultures gave, when planted, a mixture of typical "vine-leaf" colonies and of colonies which were round, raised, and with a regular unnotched edge.

Durham (1900—1901) has suggested that because of its greater relative frequency the name *B. coli communior* should be given to that form of *B. coli* which attacks cane-sugar, and that the *B. coli communis* (Escherich) which does not ferment cane-sugar should be termed the *B. coli communis verus*. He mentions that dextrose, laevulose, mannose, arabinose, galactose, and maltose, give acid or acid and gas with races which are able to ferment dextrose. Mannite and dextrin also react like dextrose in that those bacilli which give acid or gas with one of the former will do so with the latter.

W. W. Ford (1901) also considers that the most common *B. coli* is that which possesses the power of breaking up dextrose, lactose, and cane-sugar; a second variety splits up dextrose and lactose, but not cane-sugar; and a third acts on dextrose only.

Abbott (1902) thinks that the *B. neapolitanus* of Emmerich is the same organism as the *B. coli communis* of Escherich.

Hewlett (1902) states that the *B. coli communis* has been described under the names *B. cavicida* (Brieger), and *B. neapolitanus* (Emmerich).

Horrocks (1902) describes a *B. sulcatus gasoformans*, a small, slightly motile, Gram-negative bacillus which gives growths like *B. coli* except that the surface of the gelatine growth becomes wrinkled in about five days. The gas-ratio in dextrose is about 2 to 1. The amount of acid produced in litmus-whey after 7 days at 37° C. = 20 per cent. $\frac{n}{10}$ alkali.

Orlowski (1897) is of opinion that the *B. coli* is a very variable organism. He maintains,

(1) that there are many varieties in nature;

(2) that in favourable circumstances one kind may pass over into another;

(3) that such favouring conditions may be found in the animal body, as when he injected one strain he not infrequently found another in the blood and organs, while from the intestinal contents only typical *B. coli* was isolated[1].

All these authors are agreed that *B. coli* is usually a short bacillus with rounded ends; that it does not retain the dye when stained by Gram's method; that it is a non-sporing facultative anaerobe; that the growth on agar is a greyish-white layer; that the *typical* surface colony on

[1] I trust I may be pardoned for making a slight digression here to point out that such reasoning if applied to the following cases would lead to error.

From the heart-blood of a guinea-pig which had been inoculated with a pure culture of the *B. mallei* I isolated an actively motile, short rod, Gram-negative, giving ordinary grey-white growths on agar and gelatine without liquefying the latter, general turbidity in broth, no clotting in milk but preliminary slight acidity followed by marked alkalinity, and fermenting glucose, mannite, dulcite, and sorbite with the production of acid and gas, but having no effect on lactose or cane-sugar.

On another occasion two Roux bottles of nutrient agar were inoculated with a pure culture of *B. typhosus* of which the M.L.D. (guinea-pig 250) was 0·2 c.c. of a 24 hour broth culture given intraperitoneally. After 24 hours' incubation at 37° C., the growth was swept off the surface of the agar and emulsified in 3 c.c. of 0·85 % NaCl solution. One-half of this emulsion (*i.e.* 1 Roux bottle = 15—20 agar tubes) was injected subcutaneously into a guinea-pig weighing about 250 grammes. The animal died on the fourth day and from the heart-blood was isolated in pure culture a bacillus exactly like the one described above.

A similar organism appeared to be the cause of a small epidemic which broke out amongst our stock of experimental animals. The post-mortem naked-eye appearances were those of pseudo-tuberculosis rodentium. From four stock animals examined the bacillus referred to in the two previous paragraphs was isolated in pure culture from the liver.

This suggests that organisms which morphologically and culturally are indistinguishable from the *B. enteritidis* (Gaertner) may be much more widely distributed in nature than is at present recognised. It also emphasises the importance, especially in the examination of food-stuffs, of keeping animals under observation for some time previous to experiment so as to ensure that only healthy animals are used.

gelatine is a "vine leaf," thin, filmy, translucent expansion; that gelatine is never liquefied; and that glucose is always fermented with the production of acid and gas. They differ, however, with regard to the fermentation of other carbohydrates and more particularly concerning cane-sugar. This is most probably due to the fact that organisms which give distinctly different fermentative reactions have been classed together as *B. coli communis*. But an organism can be termed *B. coli communis* (Escherich) only when it gives the constant reactions given by the original organism described by Escherich. Through the kindness of Dr H. E. Durham, who gave me a sub-culture of the original microbe, I have been able to study this bacillus closely. During the years I have had it in my possession it has varied somewhat in its morphology and in its growth on agar and gelatine, but its growth in milk and its fermentation reactions have remained constant. I, therefore, cannot agree that the *B. neapolitanus* of Emmerich is identical with the *B. coli communis* of Escherich.

It may be as well to mention here that the *B. lactis aerogenes* that I have worked with is also a sub-culture of the original strain obtained through Dr Durham.

B. acidi lactici (Hüppe).

Hewlett (1902) states that it is a non-liquefying, Gram-negative bacillus which produces growths something like those of *coli* but the film on gelatine is much denser.

Kruse (1903) considers the *B. acidi lactici* (Hüppe) to be identical with the *B. lactis aerogenes* (Escherich).

Prescott (1903) isolated "lactic acid bacteria" from various cereals and meals and from waters which were apparently free from all suspicion of faecal contamination and compared them with 23 varieties of *B. coli* which had been isolated from faeces or sewage. Of 64 cultures 44 gave the same cultural reactions, and of these 25 were lactic acid bacteria and 19 were *coli*. Morphologically there was no difference and all produced the same amount of acid when tested under the same conditions.

Lehmann and Neumann (1904) describe this organism as a short oval, non-motile rod, which is Gram-negative, and does not liquefy gelatine. The growth on agar resembles that of *B. coli* but is viscid. It produces general turbidity in broth, indol in peptone solutions, clotting in milk, and acid and gas in glucose and lactose. They consider it to be identical with *B. lactis aerogenes* (Escherich), and that the

B. cavicida (Brieger) and *B. neapolitanus* (Emmerich) are most closely related to it.

B. lactis aerogenes (*Escherich*).

Theobald Smith (1895) says that one variety of *B. lactis aerogenes*, isolated from water, is only differentiated from true *B. coli* by its want of motility. The true *B. lactis aerogenes* is differentiated by the amount of gas produced and by the composition of the gas: gas-production in glucose = 80 %—100 %, $\frac{H}{CO_2} = \frac{1}{1}$ or $\frac{1}{1+}$.

Strong (1899) discussing capsulated bacilli divides them into two groups:

Group I. *B. pneumoniae* (Friedländer), B. Wright and Mallory, *B. ozaenae*, *B. sputigenes crassus*, and *B. rhinoscleromatis*. Gas production: cane-sugar 50 %, glucose 30 %, lactose 30 % or maybe none. No clotting of milk.

Group II. *B. aerogenes*, *B. capsulatus* (Pfeiffer), *B. capsulatus* (Kruse). Gas production in all sugars about the same but most in cane-sugar. Milk is clotted.

J. Rothberger (1900) describes it as a non-motile bacillus which does not form indol. It produces general turbidity in broth, acid and clot in milk, and ferments glucose with production of acid and gas. On agar the growth is whitish and slimy, or soft and whitish with a smooth margin. The surface growth of a stab gelatine culture is grey-brown, dry and slightly irregular.

Grimbert and Legros (1900) think that *B. lactis aerogenes* (Escherich), and *B. pneumoniae* (Friedländer) are the same organism because:

(1) they are non-motile;

(2) they have capsules in the blood of inoculated animals;

(3) they do not liquefy gelatine;

(4) they do not produce indol;

(5) they ferment carbohydrates and alcohols, even though the same substances are not always attacked by both bacilli.

B. pneumoniae (Friedländer) ferments dulcite, whereas the *B. lactis aerogenes* does not.

Durham (1900—1901) places *B. lactis aerogenes* among the poly-saccharide fractors, and states that it ferments dextrose, lactose, cane-sugar, and may, or may not, ferment starch and inulin. He notes that all the bacilli of this group which were tested by him gave the Voges

and Proskauer's "Kalilauge-roth-reaktion," while the bacilli of the other groups mentioned in his paper failed to do so.

Ford (1901) considers there are three forms of this organism; the most common form fermenting glucose, lactose, and cane-sugar; the second form acting on dextrose and lactose, but not on cane-sugar; and the third splitting dextrose only.

Hewlett (1902) gives the fermentation of starch and inulin as characteristics of this bacillus.

Muir and Ritchie (1902) think that this bacterium is either a variety of *B. coli* or a closely related organism. It is distinguished by its more abundant gas-production, and by its growth on gelatine and agar being thicker and whiter than that of *coli.*

Jordan (1903) describes it as a non-motile, non-liquefying bacillus which may not curdle milk until the 9th or 10th day. The gelatine colonies differ from those of *coli* in that they are less spreading, more convex and fleshy, with smooth, well-defined margins. Indol is usually produced and dextrose; lactose and cane-sugar fermented though the latter may not be acted upon.

Horrocks (1903) characterises it as a small non-motile, Gram-negative bacillus producing on agar a greyish-white tenacious growth. The gelatine colonies are thicker, and more opaque than those of *coli.* On potato the growth is coloured and gas-bubbles may appear on the surface. Milk is acidified and clotted. Indol is formed in peptone water. Glucose, lactose, cane-sugar and starch are fermented with the production of acid and gas. He also says, "Judging this organism by its power of fermenting starch, I cannot say it is frequently found in sewage polluted waters."

Paul Clairmont (1902) states that it is non-motile, gives the usual growth on agar and gelatine, does not produce indol, acidifies and clots milk, and ferments glucose, lactose, and cane-sugar.

Lehmann and Neumann (1904) consider the *B. lactis aerogenes* to be identical with the *B. acidi lactici* (Hüppe). It does not form indol, and is only differentiated from *B. coli* by its want of motility.

Taking all these opinions together the *B. lactis aerogenes* (Escherich) might be described as a non-motile, Gram-negative, non-liquefying bacillus, a facultative anaerobe, producing acid and clotting in milk, fermenting glucose, lactose, and cane-sugar and maybe also starch and inulin, and giving Voges and Proskauer's "Kalilauge-roth-reaktion" (see foot of p. 349).

B. pneumoniae (Friedländer).

Theobald Smith (1891) speaks of this organism as a bacillus giving general turbidity in broth, acid and clot in milk, not liquefying gelatine, and fermenting glucose, lactose, and cane-sugar. $\frac{H}{CO_2} = \frac{1+}{1}$.

Grimbert (1896) says that the Friedländer bacillus is found in water much more frequently than is generally imagined. Like *B. coli* it is Gram-negative, does not liquefy gelatine, and ferments lactose. It does not produce indol and is non-motile. He examined six strains and divided them into three groups:—

Group I. Ferments glucose, arabinose, raffinose, dextrin, mannite, maltose, cane-sugar, galactose, and lactose, but not glycerine, dulcite, or erythrite.

Group II. All these substances are decomposed except dulcite and erythrite.

Group III. Erythrite alone is not attacked.

Hewlett (1902) says the morphology is variable, the bacillus is non-motile, Gram-negative, and non-liquefying. Indol is not formed, milk is acidified and clotted. A copious, viscid, greyish growth appears on agar. On gelatine it forms a thick, white, shining, porcelain-like growth, and in stab gelatine culture the well-known "nail" growth.

Muir and Ritchie (1902) describe it as a short rod with blunt rounded ends, Gram-negative. It produces acid, with or without clot in milk, and a white, shiny, viscid growth on agar. The gelatine colonies are raised white discs. A stab culture shows the "nail" growth. Gelatine is not liquefied. Fermentation occurs in dextrose, lactose, maltose, dextrin, mannite, and may, or may not, occur in glycerine.

Clairmont (1902) gives the following characters. A non-motile bacillus giving the usual growths on agar, gelatine, and potato; general turbidity in broth; acid but no clot in milk; and fermenting glucose, lactose, and cane-sugar.

Lehmann and Neumann (1904) describe it as a short, round-ended, non-motile, non-sporing, Gram-negative rod, and a facultative anaerobe. The gelatine colonies are round, soft, white, and slimy looking. A gelatine culture is "nail-like." In broth it produces general turbidity, with a slimy deposit, and the fluid becomes somewhat viscid. Milk is acidified and usually no coagulation occurs within 20 days, but clotting may occur. The production of indol is slight. It is identical with or closely related to the *B. capsulatus* of Pfeiffer.

It would appear then that the *B. pneumoniae* (Friedländer) might be described as a non-motile, non-liquefying, Gram-negative, facultative anaerobic bacillus which produces acid in milk, with or without clotting, and ferments glucose, lactose, and cane-sugar. In the blood of experimental animals it is frequently seen to have a capsule.

B. cloacae (Jordan).

Theobald Smith (1895) says the *B. cloacae* is a widely-distributed, motile, strongly fermenting bacillus which gives a reaction with lactose very similar to that of *coli*. In glucose broth the gas-production amounts to 90 %—100 % of the closed arm of the fermentation tube and the ratio of H to $CO_2 = 1 : 2$ or 3.

Ford (1901) describes the typical *B. cloacae* as a motile, facultative anaerobe, fermenting dextrose, lactose, and cane-sugar, producing indol, giving general turbidity in broth, and acid and clot in milk. A second variety ferments dextrose and lactose but not cane-sugar, and does not produce indol. A third form attacks glucose only of the three sugars named, produces indol, and acidifies milk without clotting it. A fourth kind does not act upon any of the sugars, does not form indol, but clots milk. All four varieties liquefy gelatine, casein, and blood serum, and all are motile.

Irons (1902) mentions that it produces acid and clot in milk, liquefies gelatine, gives rise to acid and gas in dextrose and cane-sugar with gas ratio $H < CO_2$, and gas in lactose with a varying ratio.

Jordan (1903) writes that it is a motile bacillus fermenting dextrose and cane-sugar, which may or may not act on lactose. Indol production varies, as also does the liquefaction of blood serum and of casein. Only very rarely does it not liquefy gelatine, but the liquefaction may take 30—40 days.

Lehmann and Neumann (1904) quoting Theobald Smith (1893) describe it as a motile bacillus, liquefying gelatine, producing acid and clot in milk in 8 days, producing much gas in glucose and cane-sugar, *i.e.*, 50—95 %, $\frac{H}{CO_2} = \frac{1}{2}$. The gas-production in lactose is slow.

The *B. cloacae* (Jordan) then might be described as an actively motile, Gram-negative, facultative anaerobic bacillus, fermenting glucose and cane-sugar, and maybe lactose, producing acid and clot in milk, and which almost invariably liquefies gelatine, and may, or may not, also liquefy blood serum and casein.

The biological characters ascribed to these five organisms by the

various observers mentioned are best appreciated when tabulated for comparison :—

	Glucose	Lactose	Cane-sugar	Milk	Indol	Motility
B. coli	+	+	±	A + C[1]	+	±
B. lactis aerogenes (Escherich)	+	+	+	A + C	±	−
B. acidi lactici (Hüppe)	+	+	−	A + C	+	−
B. pneumoniae (Friedländer)	+	+	+	A ± C	∓	−
B. cloacae (Jordan)	+	±	+	A ∓ C	±	+

[1] A = Acid, C = Clot.

It is obvious that, if reliance is placed solely upon the reactions given above, these organisms may be said to be closely related: and it is not surprising that by some the *B. acidi lactici* (Hüppe) and *B. lactis aerogenes* (Escherich) should be considered identical and practically non-motile forms of the *B. coli*, or that by others the *B. lactis aerogenes* is taken to be the same organism as the *B. pneumoniae* (Friedländer). Theobald Smith differentiates these bacilli by the amount of gas produced and by the "gas ratio," and his lead is followed by most of the American bacteriologists. But these distinguishing points are not universally accepted as of value.

With the idea of, if possible, finding further points of difference I have tested the action of these and certain other organisms upon 14 carbohydrates and alcohols; have measured the amount of gas produced and the "gas-ratio," and have noted the appearance of Voges and Proskauer's reaction. The results are set out in the following table, and I desire to take this opportunity of tendering my hearty thanks to those who have so kindly sent me cultures.

It is the universal opinion that with the exception of *B. cloacae* and *B. proteus* none of these organisms liquefy gelatine, and that none of them stain by Gram's method. It has not been thought necessary to give any description of the morphology nor of the growths on agar, gelatine, or potato.

The "caustic potash red reaction[1]" was first mentioned by Voges and Proskauer (1898) in a paper on the "Bacteria of Haemorrhagic Septicaemia." They describe it thus (speaking of a Schweinepest bacillus isolated by Voges): "On the addition of caustic potash we observed a new and interesting colour reaction. If the tube be allowed to stand for 24 hours and longer at room temperature, after the addition of the potash, a beautiful fluorescent colour, somewhat similar to that of

[1] This is the reaction referred to on p. 337.

TABLE B—SHOWS CULTURAL REACTIONS OF CERTAIN GROUPS OF ORGANISMS.

Name	Obtained from	Glucose	Laevulose	Maltose	Galactose	Arabinose	Raffinose	Lactose	Cane-sugar	Mannite	Sorbite	Dulcite	Dextrin	Starch	Inulin	$\frac{H}{CO_2}$ in Glucose	Voges and Proskauer reaction
B. lactis aerogenes (Escherich)	Dr H. E. Durham	+	+	+	+	+	+	+	+	+	+	−	+	±	−	$\frac{1}{1}$ or $\frac{1}{1+}$	+
B. capsulatus (Pfeiffer)	Král	+	+	+	+	+	+	+	+	+	+	−	+	±	−	,,	+
B. pneumoniae (Friedländer)	Král through Dr Bulloch	+	+	+	+	+	+	A	+	+	+	+	+	...	...	$\frac{1+}{1}$	−
,, ,, ,,	Dr H. Spitta	+	+	+	+	+	+	+	+	+	+	+	+	...	...	$\frac{1+}{1}$	−
B. neapolitanus (Emmerich)	Král	+	+	+	+	+	+	+	+	+	+	+	+	...	...	$\frac{1+}{1}$	−
B. coli communis (Escherich)	Dr H. E. Durham	+	+	+	+	+	+	+	−	+	+	+	+	−	−	$\frac{1+}{1}$	−
B. acidi lactici (Hüppe)	Král	+	+	+	+	+	+	+	−	+	+	−	+	−	−	$\frac{1+}{1}$	−
B. enteritidis (Gaertner)	Dr H. E. Durham	+	+	+	+	+	+	−	−	+	+	+	+	−	−	$\frac{1+}{1}$	−
B. icteroides (Sanarelli)	Dr Sanarelli	+	+	+	+	A	+	−	−	+	+	A	+	−	−	,,	−
B. psittacosis (Nocard)	Pasteur Institute	+	+	+	+	+	+	−	−	+	+	+	+	...	...	,,	−
B. L. (Hume)	Dr Hume	+	+	+	+	+	+	−	−	+	+	+	+	−	−	,,	−
B. epidemic jaundice	Dr H. E. Durham	+	+	+	+	+	+	−	−	+	A	?	?	−	−	,,	−
B. hog cholera (Smith, Maryland)	Dr H. de R. Morgan	+	+	+	+	A	A	−	−	+	+	A	+	...	...	,,	−
,, (Smith, Arkansas)	,,	+	+	+	+	A	A	−	−	+	+	A	+	...	...	,,	−
B. paracolon (Day)	Dr R. T. Hewlett	+	+	+	+	+	+	−	−	+	A	A	+	...	...	,,	−
B. paratyphoid (Schottmuller A)	,,	+	+	+	+	+	+	−	−	+	+	+	+	...	...	,,	−
,, (,, B)	,,	+	+	+	+	+	+	−	−	+	+	+	+	...	...	,,	−
,, (Brion & Kayser)	,,	+	+	+	+	+	+	−	−	+	+	+	+	...	...	,,	−
B. paracolon (Le Sage)	Dr H. E. Durham	+	...	+	+	+	...	−	−	+	+	+	...	−	−	,,	−
B. cloacae (Jordan)	Král	+	+	+	+	+	+	+	+	+	+	−	+	−	−	$\frac{1}{1+}$	+
B. pyogenes foetidus	Král	A	A	A	A	A	A	A	A	A	A	A	A	...	...	...	−
B. typhi abdominalis (Eberth)		A	A	A	A	−	A	−	−	A	A	−	A	−	−	...	−
B. dysenteriae (Shiga)		A	A	A	A	−	A	−	−	−	−	−	−	−	−	...	−
,, (Flexner-Gray)	Dr H. E. Durham	A	A	A	A	A	A	−	−	A	−	−	A	−	−	...	−
B. proteus vulgaris	Král	+	...	+	+	...	...	...	+	...	−	−	...	...	...	...	...
B. pseudo-tuberculosis rodentium		A	A	A	A	...	−	−	−	A	−	−	A	...	...	...	...
B. pestis		A	A	A	A	...	−	−	−	A	−	−	A	...	...	...	...

a dilute alcoholic solution of eosin, forms in the culture fluid, particularly at the open end of the tube exposed to the air. We have investigated a few of the properties of this colouring substance, which is not produced by the action of the alkali on the sugar, and have found that it is fairly resistant to the action of the external air. After a time, however, it becomes paler, and finally gives place to a dirty greenish-brown. Prolonged boiling does not affect the intensity or *nuance* of the colour. We attempted to extract the substance by means of ether and of amyl alcohol but no trace passed into these solvents. Acetic acid changes the tint into a reddish-yellow. The isolation of the colouring substance was not successful owing to the quantity present being too small. We are compelled at present to consider this new potash reaction as specific for the bacillus in question as none of the remaining bacteria, notwithstanding the variety of culture media used, gave it. Neither does the *Bacterium coli communis* give the reaction, so that we have here a further most valuable means of differentiation from these inhibitants of the intestine." The bacillus referred to as giving this reaction was one which fermented all the carbohydrates and alcohols in which it was grown, which included glucose, lactose, cane-sugar, and mannite, and thus differed from the other varieties of swine-plague and hog-cholera which have been described.

Durham (1900—1901) refers to this reaction and states that it may appear within a few hours after adding the potash (*vide supra B. lactis aerogenes*). Freeland How, junior (1904), in "Notes on *B. coli*" refers to those organisms which produce a red colour in the closed arm when standing 24 hours after the addition of potash, and says "just what produces this red colour is not known," and "if it were possible to determine the species to which a culture characterised by this colour belongs it would be of decided advantage to bacteriologists engaged in water analysis," and "the colour is destroyed by the addition of H_2SO_4 and restored by adding an alkali."

As will be seen from the table this reaction appears to obtain only in the case of the *B. lactis aerogenes* group and *B. cloacae* group, thus entirely confirming Durham's observation that it was a point of difference between the *aerogenes* group and the *coli enteritidis* and *typhosus* groups. It also helps to distinguish the *B. pneumoniae* (Friedländer) from the *B. lactis aerogenes.*

For this test it is best to use a fermentation tube, though it may be performed in an ordinary test-tube. The colour closely resembles that of a 0·2 % solution of rosolic acid in 50 % alcohol and water.

Now if we for the present neglect the question of motility and restrict our consideration to the lactose fermenting organisms solely, a glance at the table above shows that the differentiating points have reference to:—

(1) the fermentation, or otherwise, of cane-sugar and dulcite,

(2) the gas-ratio,

(3) the appearance, or non-appearance, of the "Kalilauge-roth-reaktion" of Voges and Proskauer.

And so, just for the sake of classification in these experiments, the organisms isolated have been divided arbitrarily into four groups according as they do, or do not, ferment cane-sugar and dulcite:—

Group	1	containing	these organisms	which are	cane-sugar	–	dulcite	–
„	2	„	„	„	„	–	„	+
„	3	„	„	„	„	+	„	+
„	4	„	„	„	„	+	„	–

The – sign meaning that the organism has no action, and the + sign denoting the formation of acid and gas.

Without implying that all the organisms in Group 1 are identical one may speak of them as conforming in fermentative properties to the type of *B. acidi lactici* (Hüppe). Similarly the *B. coli communis* (Escherich) may be taken as the fermentative type of Group 2, and the *B. neapolitanus* or *B. pneumoniae* (Friedländer) as representing Group 3. As regards Group 4 it will be seen from the results given below that this group may be subdivided into:—

Sub-Group	1.	? *B. coli*[1].	No liquefaction of gelatine; absence of Voges and Proskauer reaction.
„	2.	*B. lactis aerogenes.*	No liquefaction of gelatine; presence of Voges and Proskauer reaction.
„	3.	*B. cloacae.*	Liquefaction of gelatine or casein; presence of Voges and Proskauer reaction.
„	4.	*Bacillus*?	Liquefying gelatine, and producing a yellow pigment.

[1] The pathogenic *Bacillus coscoroba* (*Ann. de l'Institut Pasteur*, 1900) conforms to this type.

Section III.

Effect of Environment upon Biological Characteristics.

It is not an uncommon experience in laboratory work to find that a stock organism has temporarily lost one or more of its characters, *e.g.*, indol or gas production, or the clotting of milk, and that repeated sub-cultivation soon causes the organism to resume its former vigour. These temporary enfeeblements are generally said to be due to "unfavourable environment," and it is supposed that a continuation of such unfavourable conditions may cause the change in characteristics to become permanent and thus give rise to a new variety. It might be said that Group 1 was in this way derived from the other groups, and therefore it was considered necessary to enter a little into this question. Jordan (1901) speaking of *coli* in river water says:—"It is quite possible that some bacilli may become so disguised by prolonged aquatic life as to be no longer recognisable by the methods used"; but "it is always possible to recover *coli* bacilli possessed of typical gas-producing qualities from sewage and from polluted river-waters that have been stored for some weeks in glass bottles in the laboratory." "In one instance we have found *colon* bacilli yielding typical gas production in a 1/1,000 dilution of sewage that had been standing in a bottle for 42 days, and in another case we have found them six months after the sewage had been collected."

Horrocks (1903) planted agar emulsions of *B. coli communis* (Escherich) on various samples of soils and examined the cultural reactions from time to time. The bacillus was isolated unchanged from a rich loam at the end of a month, from a virgin loam at the end of six weeks, from a virgin sandy soil at the end of 60 days. He comes to the conclusion that *B. coli* tends to die out in soil, and that the greatest duration of life is about 90 days. He also added emulsions of the same organism to various waters, which were, like the soils, kept at room temperature. From deep well-waters the *B. coli communis* was isolated unchanged after 31 days, but after 9 weeks and 3 months there was weakened indol formation and delayed coagulation of milk: from New River Company's water after 2 months with the same changes: and similarly from sterilised Thames water after 2 to 3 months. He comes to the conclusion that there is no evidence that *B. coli* ever becomes atypical.

Savage (1905), from experiments with *B. coli* and tidal mud, expresses the opinion that "None of my experiments bear out the idea that *B. coli* in mud, sterilised or unsterilised, alter some of their characters."

Remy (1900) grew *B. typhosus* and *B. coli* together in neutral bouillon. *B. typhosus* was isolated after 82 days of life in common. He says these conditions can modify profoundly the characters of the two organisms, the *B. typhosus* losing its sensibility to agglutinins and the *B. coli* its specific characters, *e.g.* gas and indol production.

Klotz (1904) in his study of an organism isolated by Adami and Chopin from tap-water and called by him *Bacillus perturbans* states that after passage through a rabbit there was a temporary loss of gas production in lactose and cane-sugar.

The object of the following experiments was to ascertain whether unsterilised laboratory tap-water would have any influence upon the *B. coli communis* (Escherich) in the direction of modification of its characters or of a shortening of its life.

A preliminary examination of the tap-water was necessary to obtain its average *coli* content. For this purpose the tap was allowed to run 5 to 10 minutes before filling a sterile stoppered bottle of about 200 c.c. capacity. Then 1 c.c. of the sample was pipetted off into a tube of bile-salt broth. On three occasions the rest of the sample was concentrated by filtration through a Berkefeld candle, the deposit being scraped off and put into a tube of bile-salt broth. On nine occasions, instead of concentrating the water by filtration, 10 c.c. of a 10 times concentrated bile-salt broth were added to the water in the bottle and the whole incubated at 37°C. for 24—48 hours, when a small quantity was used to inoculate a tube of bile-salt broth. When any of the broth tubes showed the production of acid or acid and gas, bile-salt agar plates were made and inoculated from the broth. After incubation at 37°C. for 24—48 hours a colony was taken and worked through glucose, mannite, lactose, cane-sugar, dulcite, agar, gelatine, broth and litmus milk. Twelve samples were in all examined. *B. coli* was found once out of 10 times in 1 c.c. and 8 times out of 11 in 200 c.c. It is interesting to note that *B. coli* was not found once in the deposit on the filter-candle, while it was found in almost every case when the concentrated broth was added to the rest of the sample in the bottle. It may, therefore, be accepted that the tap-water used did not usually contain *B. coli* in 1 c.c.

The *B. coli communis* (Escherich) was exposed to the influence of the tap-water in the following old way: A 24 hour broth culture was poured

into a sterile Pasteur candle, which was plugged with wool and suspended in a jar holding about $1\frac{1}{2}$—2 litres of tap-water. A similar arrangement was made for the *B. typhosus.* The jars were kept at room temperature in the dark in a cupboard. From time to time the jars were emptied and filled with fresh water, and the contents of the candles tested for the presence of *B. coli* and *B. typhosus* respectively. Thus:

3rd day.	Water changed.
5th ,,	,, ,,
6th ,,	*B. coli* found inside but not out.
	B. typhosus found inside but not out.
10th ,,	*B. coli* found inside but not out. Water changed.
19th ,,	Water changed.
20th ,,	*B. coli* and *B. typhosus* present inside the candles.
24th ,,	,, ,, ,, ,, ,, and water changed.
27th ,,	On this day gelatine plates were poured from the contents of the candles and these showed the presence of innumerable extraneous organisms.
	B. coli and *B. typhosus* found inside in one loopful.
50th ,,	*B. coli* and *B. typhosus* inside in 1 c.c. but not outside.
52nd ,,	Water changed.
77th ,,	*B. coli* inside but not out. *B. typhosus* apparently dead. Water changed.
81st ,,	No *B. typhosus* found in the whole of the contents of the filter-candle.
123rd ,,	*B. coli* found inside in 1 c.c.
266th ,,	Water changed.
267th ,,	*B. coli* in 1 c.c. inside.
281st ,,	The jar was emptied several days ago and has been allowed to remain so, and the candle to drain itself dry.
310th ,,	The filter-candle is empty and dry. About 300 c.c. of cold boiled distilled water poured into the filter.
311th ,,	*B. coli* found in 1 c.c. of the water in the filter. The fermentation of dulcite took a day or so longer than usual, otherwise the organism was unchanged.
329th ,,	The filter has been gradually drying. There is no water in it to-day.
357th ,,	10 c.c. of sterile water were poured into the filter. It was all absorbed. Then 20 c.c. more were put in, washed round and 2 c.c. used for testing. *B. coli* was recovered unchanged.
358th ,,	10 c.c. of sterile water were put into the filter and washed round. Then 1 c.c. was taken out and spread over the surface of a bile-salt lactose-agar plate. This was done with three plates which were incubated at 42° C.; one plate yielded 2 colonies, another 16 and the third none. One of the colonies was taken and on working out gave *B. coli* unchanged.
388th ,,	The filter was washed inside with 20 c.c. of sterile water, 1 c.c. of which was tested for the presence of *B. coli*. The result was negative.

The *B. coli* was not recovered from the *B. typhosus* filter in one single instance and this may, therefore, be considered as a control to the *B. coli* candle.

A further control experiment was performed as follows: The same filter-candle that had been used for the *B. coli* in the previous experiment was cleaned, plugged and sterilised. It was then suspended as before in a jar of tap-water and 10 c.c. of sterile nutrient bouillon was poured into it. The water in the jar was changed from time to time as before and the broth inside the filter tested for *B. coli*. Thus:

2nd day.	No growth whatever obtained in ordinary bouillon from 1 c.c. of the liquid inside the filter.
4th "	Water changed.
15th "	Water changed. Growth but not of *B. coli* from 1 c.c. of the liquid inside the filter.
23rd "	No *B. coli* inside.
29th "	Water changed. No *B. coli* inside.
48th "	Water changed. No *B. coli* inside.
107th "	Water changed.
125th "	No *B. coli* inside.
226th "	Water changed.
239th "	No *B. coli* inside.

The experiment was discontinued on this day as it was thought that it had been in progress for a sufficiently long time to prove that under the conditions of the experiment any *B. coli* which might be present in the tap-water would not be likely to grow through the filter and multiply to such an extent as to be present in every cubic centimetre of the inner liquid. We may conclude then that none of the *B. coli* isolated in these experiments came from the tap-water filling the jar but they were descendants of the *B. coli communis* culture originally put into the filter-candle.

It is justifiable, therefore, to assert that in this case the *B. coli communis* (Escherich) retained all its characters unchanged after an exposure of 358 days to what was a changing and unfavourable environment, a result distinctly opposed to the idea that *B. coli communis* ever becomes atypical: and tending to confirm the work of Horrocks and Savage. The result also affords presumptive evidence that one group is not derived from another by loss of characteristics on the part of some of its members.

Section IV.

Effect of Preliminary Incubation in a Liquid Medium.

In these experiments if any particular organism predominated it might be said that the results of the examination did not represent the true state, as, though all the various organisms might be present in equal numbers, the preliminary incubation in broth might allow one variety to overgrow the others. It became necessary, therefore, to make a few experiments to ascertain the effect of the preliminary incubation upon mixtures of certain known organisms.

Inoculations were, therefore, made from old gelatine cultures of *B. coli communis* (Escherich), *B. acidi lactici* (Hüppe), *B. cloacae* (Jordan), *B. lactis aerogenes* (Escherich), and C. 46 (a yellow liquefier, isolated from cow-dung) into the same tube of bile-salt mannite broth, which was incubated at 37° C. Next day surface cultivations were made on bile-salt lactose agar plates. Both broth and plates were placed at 37° C., and next day plates were again made from the broth. We have thus plates made from a mixture after 24 and 48 hours' preliminary incubation in a broth. After each set of plates had been incubated for about 24 hours, 12 colonies were taken from each set and sub-cultured in cane-sugar and dulcite. The results were:—

	B. acidi lactici	*B. coli*	*B. lactis aerogenes* *B. cloacae*	Total
24 hour plates	2	—	10 (1 was C. 46)	12
48 ,, ,,	11	1		12

The above experiment was repeated with the difference that nutrient broth cultures about one week old were used to inoculate the tube of bile-salt broth; and 10 colonies only were taken from each set of plates. Result:—

	B. acidi lactici	*B. coli*	*B. lactis aerogenes* *B. cloacae*	Total
24 hour plates	—	—	10	10
48 ,, ,,	2	—	8	10

A third experiment was made on similar lines using a mixture of *B. coli communis* and *B. acidi lactici* only. It was considered enough to take 6 colonies from each set of plates. Result:—

	B. coli	*B. acidi lactici*	Total
24 hour plates	1	5	6
48 ,, ,,	—	6	6

Further, the *B. lactis aerogenes* and *B. cloacae* were inoculated into the same tube of bile-salt mannite broth, and incubated at 37° C. At the end of 24 hours and 72 hours plates were made from the broth on bile-salt lactose agar. After 24 hours at 37° C. colonies were taken from the plates, sub-cultured into broth and examined. Results:—

	B. lactis aerogenes	*B. cloacae*	Total
24 hour plates	9	1	10
72 ,, ,,	10	0	10

A repetition of this experiment in which plates were made after the broth had been incubated at 37° C. for 24, 48, and 72 hours yielded

		B. lactis aerogenes		*B. cloacae*
24 hours	in the proportion of	4	to	1
48 ,,	,, ,,	30—40	to	1
72 ,,	,, ,,	1	to	0

The preliminary incubation might, therefore, possibly alter the relative proportions by favouring the growth of the fourth (*lactis aerogenes*) group; but if the *B. coli communis* is found, then it is not at all likely that any of the other organisms have been overwhelmed.

Section V.

As previously mentioned, these experiments were instituted for the purpose of ascertaining the relative frequency of occurrence of certain lactose-fermenting bacilli, but they resolved themselves into a search for the *B. lactis aerogenes*. The change came about in this way. My colleague, Dr A. Harden, was conducting an investigation into the chemical action of these organisms and I passed cultures of the organisms isolated over to him. As organism after organism of the fourth group (p. 352) with which I supplied him failed to satisfy his tests for a *B. lactis aerogenes* I was led to pay more attention to this organism than to others. But there was also underlying these experiments the idea that a more extended use of carbohydrates and alcohols might possibly bring to light differences between organisms of various origins. It is on this account that the experiments seem somewhat disconnected and that the references to previous work touch upon such a variety of points.

All the organisms isolated had, unless otherwise stated, the following characters in common:—

They were non-sporing, Gram-negative, facultative anaerobic bacilli,

gave grey-white growths on agar and gelatine, without liquefaction of the latter; produced acid and clotting in milk; general turbidity, with formation of indol, in broth; and fermented glucose, lactose, and mannite with the production of acid and gas. Their action on cane-sugar and dulcite is mentioned in connection with each experiment.

(*a*) *Human faeces.*

Of those who in recent years have examined human faeces there may be mentioned:—

Hans Hammerl (1897) who from his investigations came to the conclusion that whether the food is sterile or not, whether it is purely vegetable or mixed, it has no great influence upon the number of living bacteria in the faeces. The only thing noticed was that when the food was sterile the usual saprophytes commonly found in man's surroundings disappeared from the stools and almost only *B. coli* and *B. lactis aerogenes* were present and fluorescing liquefiers were absent. W. Booker (1897) studying the summer diarrhoea of infants found *B. proteus vulgaris, Streptococcus, B. coli* and *B. lactis aerogenes* to be the most common organisms. Péré (1898) mentions that the *B. coli* of sucklings attacks cane-sugar, dulcite and glycerine, whereas the *B. coli* of adults does not. Horrocks (1901) compared 80 *coli* from typhoid stools with 70 *coli* from normal faeces but could not find any constant characters which distinguished the one kind from the other. Hellström (1901) examined the faeces of new-born children, taking his samples with every aseptic precaution. He found the first meconium was sterile; later air organisms appeared; and later still these latter disappeared and were replaced by the usual intestinal organisms, especially the *B. coli* and *B. lactis aerogenes.* Ford (1901) states that *B. lactis aerogenes* is specially found in the small intestine but may pass beyond the iliocaecal valve, where, coming in contact with *B. coli*, it disappears. He also says that *B. cloacae* abounds in the caecum. Kohlbrügge (1901) considers that the small intestine either has no bacteria peculiar to itself or they belong to the *B. coli* group and that the caecum exhibits the typical *B. coli.* Brown (1903) examined the stools of 21 cases of asylum dysentery and isolated the

B. enteritidis (Gaertner)	in 1 case.
B. aerogenes	,,
B. acidi lactici	,,
B. xerosis	,,

Houston (1902—1903) found there were more than 100 millions but less than 1,000 millions of *B. coli* per gramme of normal human faeces. He isolated and worked out the reactions of 100 of these organisms. As regards indol 99 % were positive; in milk 92 % produced acid and clotting, 8 % acid only: in dulcite 55 % gave acid and gas, while in cane-sugar media 8 % were strongly positive, 22 % feebly positive, 35 % doubtfully negative, and 35 % negative.

We might then expect to be able to isolate *B. coli* and *B. lactis aerogenes* from every sample of faeces. My own experiments do not justify this expectation as regards the latter organism as will be seen from the details of the results.

Just in case it should happen that a variation in the organisms isolated might be caused by the particular kind of broth used for the preliminary incubation, a sample of normal human faeces was taken and a loopful placed in each of 8 tubes of broth. The inoculated tubes were put into the warm incubator till the next day when gelatine plates were poured from each tube. From each set of plates 6 colonies were taken and sub-cultured. Of the 48 organisms thus isolated, 12 grew badly, or not at all, at 37° C. The remaining 36 were bacilli having the characteristics already mentioned. The action on cane-sugar and dulcite is given in the following table, which also gives the number isolated from each set of plates:

Medium used for preliminary incubation	Group I. Cane-sugar − Dulcite −	Group II. Cane-sugar − Dulcite +	Group III. Cane-sugar + Dulcite +	Group IV. Cane-sugar + Dulcite −	Total
Bile-salt-inulin-broth	2	3	...	...	5
,, glycerine-broth	1	2	...	...	3
,, starch ,,	1	3	...	...	4
,, dulcite ,,	...	4	...	...	4
,, cane-sugar-broth	4	—	...	...	4
,, mannite ,,	3	3	...	...	6
,, lactose ,,	2	4	...	...	6
,, glucose ,,	2	2	...	...	4
Total	15	21			36

It is noteworthy that not one of the 36 organisms fermented cane-sugar, glycerine, inulin, or starch. One produced in starch-broth an action which was of such a doubtful character that it has been considered negative. Four were distinctly motile; the rest did not show any motility under the conditions of observation. The growth on potato varied from "invisible" to an abundant yellowish-brown growth. From

this experiment it may fairly be concluded that the carbohydrate or alcohol in the medium used for preliminary incubation apparently has no selective action upon the organisms growing in it.

In the next experiment samples from the stools of persons in an ordinary state of health were examined. There were 9 samples in all taken from 3 persons; from "*A*" one sample, from "*B*" two samples, and from "*C*" six samples spread over several months. The method adopted consisted in a preliminary incubation in a bile-salt broth followed by agar plates except in the case of the 1st, 3rd, and 4th samples when gelatine plates were used. The results are set out in the following table:

Sample	Group 1.	Group 2.	Group 3.	Group 4.	Total number isolated
	Cane-sugar − Dulcite −	Cane-sugar − Dulcite +	Cane-sugar + Dulcite +	Cane-sugar + Dulcite −	
1	2	1	...	3*	6
2	12	...	...	...	12
3	...	1	2	...	3
4	2	...	2	1**	5
5	...	2	4	4	10
6	4	1	2	3	10
7	...	10	...	...	10
8	3	2	6	1	12
9	10	...	...	...	10
Total	33	17	16	12	78

* All three organisms in Group IV. were of the *B. lactis aerogenes* type.
** The organism in Group IV. was of the *B. lactis aerogenes* type.

Thus we find all four groups present in ordinary faeces with a majority in favour of the two first groups. It is striking that such a small number of the *B. lactis aerogenes* should be isolated by this method considering that the organism grows well on these media and is accepted as being so universally present in faeces. Sample No. 9 was from a liquid stool following the administration of Hunyadi-Janos Water. In the case of sample 8 plates were made after incubation periods of 1, 2, 4, and 6 hours with the idea of ascertaining whether any particular organism preponderated at these times. There was no difference to be made out between the organisms isolated from these plates and those isolated in the usual way after 18—24 hours' preliminary incubation.

The next step was to ascertain whether there was any difference in the kind and relative frequency of the organisms isolated from the stools of cases of diarrhoea or enteric fever which would enable us to

distinguish them from those of normal faeces. The method was the same as before, agar plates being made in every instance. Each sample was from a separate case.

Sample	Group 1. Cane-sugar − Dulcite −	Group 2. Cane-sugar − Dulcite +	Group 3. Cane-sugar + Dulcite +	Group 4. Cane-sugar + Dulcite −	Total	Case
1	...	1	3	4	8	Diarrhoea; 10 organisms isolated, 2 were of the *B. pyogenes foetidus* type.
2	4	4	2	...	10	Diarrhoea.
3	...	10	...	...	10	Diarrhoea.
4	8	1	...	...	9	Convalescent from enteric fever: 1 month after commencement of attack: *B. typhosus* abundantly present.
5	8	1	1	...	10	Enteric fever.
6	1	6	3	...	10	Enteric fever.
Total	21	23	9	4	57	

These results tend to confirm those of Horrocks in showing that there is no marked difference in the character of the organisms of normal and those of diarrhoeic or enteric stools. There seems, however, a distinct preponderance of the first groups. We have again to note the absence of *B. lactis aerogenes*.

In the next experiment the faeces of one person were examined every day for a week. The diet taken during that time was ordinary mixed diet but no unboiled milk was taken. The result of the seven samples is set out below:

Sample	Group 1. Cane-sugar − Dulcite −	Group 2. Cane-sugar − Dulcite +	Group 3. Cane-sugar + Dulcite +	Group 4. Cane-sugar + Dulcite −	Total number isolated
1	9	1	...	...	10
2	1	...	1	8	10
3	3	...	2	5	10
4	1	8	1	...	10
5	...	6	4	...	10
6	...	7	3	...	10
7	...	10	...	...	10
Total	14	32	11	13	70

In this case the only point of interest lies in the relatively small number of the first group present. This result should be considered in connection with the examination of cats' faeces (p. 367).

If we take the result of the examination of human faeces as a whole, we find that 23 samples of faeces have been analysed from which 241 lactose-fermenting organisms have been isolated, of which 83, or 34 %, belong to the first group, 93 or 38 % belong to the second group, 36 or 15 % belong to the third, and 29 or 12 % belong to the fourth. Only 4 organisms out of 241 were shown definitely to belong to the *B. lactis aerogenes* type. The *B. cloacae* was not isolated in one instance. It must be noted that the Voges and Proskauer reaction was not made use of in these experiments. The cane-sugar fermenting bacilli numbered 65 while the non-cane-sugar fermenters reached a total of 176, a proportion which is opposed to the statement that the cane-sugar fractors are the more numerous organisms.

(*b*) *Animal faeces.*

Up to the present no differentiating points have been discovered which would enable us to distinguish between the faecal bacteria of the various domesticated animals, or between these and those of human excreta, similar organisms having been found in all. Thus Smith (1891) found in swine faeces bacilli which gave general turbidity in broth, acid and clot in milk, did not liquefy gelatine, and fermented glucose, cane-sugar and lactose. The gas-reactions were similar to those of the *B. pneumoniae* (Friedländer). Dyer and Keith (1894) found no difference between human and animal *coli*. In horse-dung the most common organism was *B. equi intestinalis* which does not grow on agar at 20° C., reddens litmus-lactose-agar, and clots milk but produces no gas. Lembke (1896) examined dog faeces under varying diets, *e.g.* bread, meat, fat, and mixed diets. The experiments extended over 65 days and 81 samples of faeces were examined. Only one species of organism was always present, *i.e. B. coli*. He describes a

> *B. coli anindolicum*: a motile bacillus which produces no indol-gas in glucose 30 %; $\frac{H}{CO_2} = 5/3$; lactose 30 %, $\frac{H}{CO_2} = 4/3$; milk is acidified and clotted.

and

> *B. coli anaerogenes*: a non-motile indol-producing bacillus, acidifying and clotting milk but producing only acid in glucose and lactose.

Horrocks (1902) examined the dejecta of flies, sheep, and rabbits

and found typical *B. coli communis* to be present in all of them. He cites Moore and Wright as having examined 45 cultures of *B. coli* isolated from domesticated animals and having found 23 to be negative towards cane-sugar and 22 positive. E. Heinick (1903) examined bacteriologically the intestinal canal of 23 pigs. Of the organisms present only *B. coli* and *B. lactis aerogenes* occurred regularly. *B. coli* were the most numerous by far and were identical with human *coli*. *B. lactis aerogenes* occurred in almost equal numbers. *B. coli* were most numerous in the small gut and caecum, but were often overgrown in the rectum by *B. lactis aerogenes.* This last statement is just the contrary to Ford's conclusion concerning the fate of *lactis aerogenes* in the human caecum.

My own experiments include the examination of five samples of faeces from the horse and six from the cow and calf. The methods used were the same as in the case of human excreta and the results are given in the following tables:

Sample	Group 1.	Group 2.	Group 3.	Group 4.	Total
	Cane-sugar − Dulcite −	Cane-sugar − Dulcite +	Cane-sugar + Dulcite +	Cane-sugar + Dulcite −	
Horse 1	7	2	1	...	10
,, 2	4	2	4	...	10
,, 3	2	1	7	...	10
,, 4	1	2	6	2	11
,, 5	...	8	2	...	10
Total	14	15	20	2	51
Cow 1	2	...	8	...	10
,, 2	2	4	2	2	10
Calf 3	...	...	10	...	10
,, 4	3	1	2	3	9 [1]
,, 5	1	3	1	...	5
,, 6	...	4	...	Yellow liq.	4 [2]
Total	8	12	23	5	48
Total from the 11 samples	22	27	43	7	99

[1] The 10th was a *B. cloacae*.

[2] The 5th was a lactoso-fractor, liquefying gelatine and producing a yellow pigment.

In both kinds of dung the members of the third group come into greater prominence than in the case of human faeces. Beyond this there is no point of difference. The organisms give just the same reactions as those of human origin. None of the bacilli of Group 4 were identified as being of the *B. lactis aerogenes* type.

As human enteric stools had been examined there was interest in examining the faeces of animals that had been inoculated with typhoid toxine, which I was enabled to do through the kindness of Dr Allan Macfadyen.

Rabbit No. 1. Intravenous injection of sterile typhoid bacillus cell-juice. Diarrhoea, death.

Rabbit No. 2. Intravenous injection of sterile typhoid bacillus cell-juice. Diarrhoea, death in 5 days.

Rabbit No. 3. Intravenous injection of chloroformed typhoid bacillus cell-juice, death in 3 days.

Sample	Group 1. Cane-sugar	Dulcite	Group 2. Cane-sugar	Dulcite	Group 3. Cane-sugar	Dulcite	Group 4. Cane-sugar	Dulcite	Total
	−	−	−	+	+	+	+	−	
No. 1	1		...		1		8		10
2	...		...		...		10		10
3	1		8		...		1		10
Total	2		8		1		19		30

An examination was also made in the case of a monkey which developed diarrhoea and died after being inoculated by Dr Macfadyen with typhoid bacillus cell-juice. The result was:

Group 1	0
,, 2	2
,, 3	1
,, 4	6
Total	9 organisms

and two other organisms which gave the reactions of *B. pyogenes foetidus*. There was isolated at the same time a short non-motile Gram-negative bacillus which gave grey-white growths on agar and gelatine without liquefaction, produced general turbidity in broth, acid only in milk, and in glucose and mannite, while lactose, cane-sugar, and dulcite were unaffected. This organism was clumped in a dilution of 1 in 200 by a dysentery serum which agglutinated its own bacillus up to 1 in 800.

(c) *Milk.*

Four samples of milk were examined with the object of ascertaining whether (1) the *B. acidi lactici* (Hüppe) or the *B. lactis aerogenes* (Escherich) was present in relatively larger numbers than the organisms of the other groups, and (2) whether, considering that milk is especially

liable to contamination by cow-dung, the lactose fermenters would be present in proportions similar to those of cow faeces. The examination was conducted in the same way as before.

Sample	Group 1.	Group 2.	Group 3.	Group 4.	Total
	Cane-sugar − Dulcite −	Cane-sugar − Dulcite +	Cane-sugar + Dulcite +	Cane-sugar + Dulcite −	
1	3	4	3	...	10
2	5	1	1	3	10
3	5	1	4	...	10
4	...	...	...	10	10
Total	13	6	8	13	40

There is nothing about this result which resembles that of the faeces of the horse or cow. It might be said almost to be just the reverse, Groups 1 and 4 predominating instead of Nos. 2 and 3.

The percentage of organisms of Group 1 is almost the same as that in human faeces but as regards Group 4 none of the organisms isolated were identified as of the *B. lactis aerogenes* type: one cannot, however, be quite certain upon this point as Voges and Proskauer's reaction was not employed.

The slight differences which appear to exist between the various materials examined are best shown by putting the percentages together in one table.

	Group 1	Group 2	Group 3	Group 4
Human faeces	34 %	39 %	15 %	12 %
Horse and cow	22	27	43	7
Milk	32·5	15	20	32·5
Typhoid (rabbit)	7	27	3	63
Cat (on special diet)	11	41	22	26

If human and animal faeces are considered together we find that some 500 organisms have been isolated of which about 24 % belong to Group 1, 37 % to Group 2, 22 % to Group 3, and 18 % to Group 4: thus showing that organisms of the *B. coli communis* type are about 30 % more numerous in faeces than any of the other groups. In this calculation I am anticipating somewhat by including the results of the examination of cat faeces.

(*d*) *Examination of animal faeces when the animal was fed on a partially sterilised diet.*

Certain rough experiments which have not been described here suggested that the presence of certain organisms in faeces might possibly

be associated with the consumption of certain food-stuffs. A cat was therefore confined in a cage and fed upon cooked food. As soon as the food had been boiled it was put while still "boiling hot" into the feeding trough. The drinking water was treated in the same way. No attempt was made to ensure absolute sterility of the food and the surroundings of the animal. Each consecutive stool was examined by the method previously used. The results are detailed below:

	Group 1.		Group 2.		Group 3.		Group 4.			
Sample	Cane-sugar	Dulcite	Cane-sugar	Dulcite	Cane-sugar	Dulcite	Cane-sugar	Dulcite	Total organisms isolated	
	−	−	−	+	+	+	+	−		
1	4		3		3		...		10	First stool passed after commencement of diet of boiled meat and water.
2	3		6		1		...		10	Faeces firm and dark coloured, sometimes almost black.
3	8		2		...		...		10	
4	...		...		1		9		10	
5	...		...		10		...		10	
6	...		...		10		...		10	This day diet changed to boiled milk.
7	...		...		1		9		10	First stool since change of diet, blackish-brown and very firm.
8	...		...		...		10		10	White, soft and slimy faeces.
9	...		...		2		8		10	Greyish or yellowish faeces of a soft slimy or mucoid consistency.
10	...		10		...		...		10	
11	...		9		1		...		10	
12	...		10		...		...		10	Today diet changed to boiled meat and water.
13	...		9		1		...		10	First faeces since change of diet, very firm and dark grey in colour: B. 28 added to food.
14	...		9		1		...		10	Formed greyish-brown stool.
Total	15		58		31		36		140	
15	...		1		4		5 (B. 28)		10	Pultaceous yellowish-brown faeces.
16	...		6		...		2 (B. 28)		8	

The animal was then given its freedom in the yard and put on ordinary diet. About two months later a sample was examined and yielded 10 organisms of Group 3. The animal was then again put on a diet of boiled meat and water and after a week or two of this diet a sample analysed gave 10 bacilli belonging to Group 2.

Bacillus 28 which was added to the food towards the end of the experiment was an organism of the *B. lactis aerogenes* type which had been isolated from human faeces in the earlier experiments. This organism appeared in the cat's faeces 24 hours after administration. No organisms of this type had been found in any of the previous

14 samples, but the passage of B. 28 through the intestines and its appearance in the faeces shows that if organisms of this type had been present they would have been found by the method used. Worked out in percentages the numbers read:

Group 1, 11 %	Group 2, 41 %
Group 3, 22 %	Group 4, 26 %

showing a distinct preponderance of organisms of the fermentative type of the *B. coli communis*.

The early and complete disappearance of all organisms of the first group and the entire absence of the *B. lactis aerogenes* is most remarkable.

(*e*) *Examinations at successive intervals of time, of a mixture of tap-water and human faeces.*

Bearing in mind that the *B. lactis aerogenes* grows well on bile-salt media and that it is stated to be commonly present in faeces, it is remarkable how small a number of these organisms has been isolated during the course of the previous experiments. It was thought that this might be possibly due to their being present originally in numbers which are small compared with the *B. coli*, and that they might come more into evidence if they had more time to multiply. The following experiment was performed with this idea in view and also for the purpose of ascertaining the changes, if any, which occur in the relative proportions of the four groups when faeces are diluted with water and kept for some time in a flask in the dark at room temperature. A litre flask was filled with tap-water, steamed in Koch's sterilizer for about half-an-hour, allowed to cool, and then inoculated with an amount of human faeces sufficient to cause a distinct turbidity of the whole contents of the flask. About 0·1 c.c. of the mixture was at once rubbed over the surface of three bile-salt lactose-agar plates. After 48 hours' incubation at 42° C., there was only 1 colony on the three plates. The two sterile plates were re-inoculated from the flask (= sample 1), and 0·5 c.c. from the flask was put into bile-salt mannite broth from which after 24 hours' incubation plates were made (= sample 2). On the 12th day 1 c.c. from the flask (previously well shaken) was put into the bile-salt broth and subsequently plated. This formed sample 3:—

Sample	4	was	taken on the	23rd	day,
,,	5	,,	,,	30th	day,
,,	6	,,	,,	37th	day,
,,	7	,,	,,	44th	day.

In all 75 organisms were isolated and the results are shown in the following tables, the first giving the number in each group which occurred in each sample, and the second giving some details of all the organisms isolated. All the organisms fermented glucose, lactose and mannite with the formation of acid and gas. For obvious reasons more attention was paid to the members of the 4th group than to the rest.

Sample	Group 1.		Group 2.		Group 3.		Group 4.		Total number isolated
	Cane-sugar	Dulcite	Cane-sugar	Dulcite	Cane-sugar	Dulcite	Cane-sugar	Dulcite	
	−	−	−	+	+	+	+	−	
1	1		5		2		4		12
2	5		2		1		3		11
3	...		3		...		4		7
4	...		10		...		...		10
5	...		1		1		8		10
6	...		5		5		...		10
7	...		11		1		3		15
Total	6		37		10		22		75

The gas percentages and ratios (Table C, next page) were noted after 3—4 days' incubation except in the case of sample 4 when 6 days were allowed for growth. The numbers given are not necessarily those yielded by the organism when first isolated. Thus:—

No.	1st Test Gas	1st Test $\frac{H}{CO_2}$	2nd Test Gas	2nd Test $\frac{H}{CO_2}$	3rd Test Gas	3rd Test $\frac{H}{CO_2}$
21	35 %	1/1	70 %	2/3	80 %	2/3
13	25	4/1	12	5/1	50	2/1
17	40	2/1	20	3/1	50	2/1
27	17	2/1	80	2/3	80	1/2
28	12	4/1	80	1/1	60	1/1
29	25	2/1	70	1/2	50	2/3
30	40	1/1	80	2/3	80	1/2
41	85	2/3	55	4/3		
42	85	2/3	60	1/1		
43	90	1/2	60	1/1		
44	80	2/3	55	1/1		
45	85	1/2	55	1/1		
46	50	3/2	50	1/1		
47	35	11/1	60	2/1		
48	45	4/1	55	3/1		
61	75	2/3	55	1/1		
62	65	2/3	40	1/1		
63	55	2/3	50	1/1		
9	80	2/3	40	1/1		
10	80	2/3	55	4/3		
11	75	1/2	55	1/1		
12	70	2/3	60	1/1		

TABLE C—SHOWING CERTAIN REACTIONS OF THE ORGANISMS ISOLATED FROM A MIXTURE OF TAP-WATER AND FAECES.

		Cane-sugar	Dulcite	Starch	Inulin	Gas %	$\frac{H}{CO_2}$	Voges & Proskauer's reaction	Motility	Indol	Milk	Gelatine
Sample 1	1	–	+			25	4/1			–	A & C	Non-liq.
	2	+	+			15	4/1			–	,,	,,
	3	–	–			20	4/1			–	,,	,,
	4	–	+			25	4/1			+	,,	,,
	5	–	+			35	2/1			–	,,	,,
	6	+	+			20	4/1				,,	,,
	7	–	+			35	2/1			+	,,	,,
	8	–	+			25	9/1			+	,,	,,
	9	+	–	–	–	80	2/3	+	+	–	Casein liq.	Liq.
	10	+	–	–	–	80	2/3	+	+	–	,,	,,
	11	+	–	–	–	75	1/2	+	+	–	,,	,,
	12	+	–	–	–	70	1/2	+	+	–	,,	,,
Sample 2	13	+	–	–	–	25	4/1	–	–	–	A & C	Non-liq.
	14	–	–			25	12/1			+	,,	,,
	15	–	–			20	11/1			–	,,	,,
	16	–	+			20	7/1			+	,,	,,
	17	+	–	–	–	40	2/1	–	–	–	,,	,,
	18	+	+			30	2/1			–	,,	,,
	19	–	–			30	2/1				,,	,,
	20	–	+			10	1/0			–	,,	,,
	21	+	–	–	–	70	2/3	+	+	+	Casein liq.	Liq.
	22	–	–			30	4/1			–	A & C	Non-liq.
	23	–	–			23	4/1			–	,,	,,
Sample 3	24	–	+			10	1/0			+	,,	,,
	25	–	+			25	5/1			–	,,	,,
	26	–	+			10	1/0			+	,,	,,
	27	+	–	–	–	80	1/2	+	+	+	Casein liq.	Liq.
	28	+	–	–	–	80	1/2	+	+	+	,,	,,
	29	+	–	–	–	70	1/2	+	+	+	,,	,,
	30	+	–	–	–	80	1/2	+	+	–	,,	,,
Sample 4	31	–	+			20	7/1				A & C	Non-liq.
	32	–	+			20	8/1				,,	,,
	33	–	+			30	4/1				,,	,,
	34	–	+			20	7/1				,,	,,
	35	–	+			25	18/1				,,	,,
	36	–	+			20	8/1				,,	,,
	37	–	+			30	11/1				,,	,,
	38	–	+			25	4/1				,,	,,
	39	–	+			30	3/1				,,	,,
	40	–	+			25	6/1				,,	,,

Sample 5	41	+	−	−	−	85	2/3	+	+	−	Casein liq.	Liq.
	42	+	−	−	−	85	2/3	+	+	−	,,	,,
	43	+	−	−	−	90	1/2	+	+	−	,,	,,
	44	+	−	−	−	80	2/3	+	+	−	,,	,,
	45	+	−	−	−	85	1/2	+	+	+	,,	,,
	46	+	−	−	−	50	3/2	+	+	+	A & C	,,
	47	+	−	−	−	35	11/1	−	−	+	,,	Non-liq.
	48	+	−	−	−	45	4/1	−	−	−	,,	,,
	49	+	+			40	1/0			−	,,	,,
	50	−	+			40	15/1			+	,,	,,
Sample 6	51	−	+			40	4/1			+	,,	,,
	52	+	+			40	1/0			+	,,	,,
	53	−	+			22	8/1			−	,,	,,
	54	+	+			18	7/1			+	,,	,,
	55	+	+			25	4/1			+	,,	,,
	56	+	+			18	8/1			+	,,	,,
	57	+	+			25	7/1			+	,,	,,
	58	−	+			25	4/1			−	,,	,,
	59	−	+			35	5/1			+	,,	,,
	60	−	+			45	12/1			+	,,	,,
Sample 7	61	+	−	−	−	75	2/3	+	+	−	Casein liq.	Liq.
	62	+	−	−	−	65	2/3	+	+	−	,,	,,
	63	+	−	−	−	55	2/3	+	+	−	,,	,,
	64	−	+			30				+	A & C	Non-liq.
	65	−	+			40	4/1			+	,,	,,
	66	−	+			40	3/1			−	,,	,,
	67	−	+			30	6/1			+	,,	,,
	68	−	+			45	12/1			−	,,	,,
	69	−	+			45	6/1			−	,,	,,
	70	−	+			30	5/8			−	,,	,,
	71	+	+			45	8/1			+	,,	,,
	72	−	+			50	9/1			−	,,	,,
	73	−	+							+	,,	,,
	74	−	+							+	,,	,,
	75	−	+							−	,,	,,

Some organisms were tested four or more times but it is considered unnecessary to give these results. It is apparent that the gas-reaction given by these organisms may vary considerably, and that on occasion an excess of hydrogen over CO_2 is not enough to exclude a bacillus from the *cloacae* group. Voges and Proskauer's reaction, however, was given even where the gas ratio was not typical.

None of the above organisms of the fourth group liquefied blood serum in three weeks.

In litmus milk they all produced acid and caused clotting. Later, it was noticed that the surface layer of some was turning blue and the casein was being dissolved. After 3 months' growth (mostly at room temperature) the surface layer of most was blue, then a layer of turbid yellowish fluid, and at the bottom yellowish softened clot. There was distinct solution of the casein. The only exceptions were Nos. 13, 17, 46, 47, and 48. In the case of 13, 17, 47, and 48 the clot was quite firm, pink above and decolorised below. There was very little fluid. In 46 the clot was pink above and decolorised below but it was not quite so firm as the others and there was more liquid in the tube. There was not, however, any apparent solution of the casein.

In 6 % gelatine none of these organisms caused liquefaction in 5—6 weeks but when examined at the end of three months it was found that with the exception of 13, 17, 47, and 48 all had caused about half an inch of liquefaction. There was no liquefaction whatever in the case of the four bacilli mentioned. Of the 75 organisms isolated we find 22 belonging to the fourth group, and of these 18 are members of the *B. cloacae* group, one differing from the rest in not liquefying casein, and the remaining four may for the present be classed as *B. coli.* We are again struck by the absence of *B. lactis aerogenes,* and this is all the more remarkable from the fact that this sample of faeces was supplied by a person from whose dejecta *B. lactis aerogenes* had been isolated in a previous experiment. Just as in the case of cat faeces, the members of Group 1 cease to appear quite early in the experiment. After 7 weeks' exposure to the conditions of the experiment the second group still occurs in undiminished numbers.

Section VI.

Fermentation of Starch and Inulin.

The fermentation of starch or inulin appears to be a property not commonly possessed by these organisms, for 123 (60 from human, 17 from horse, and 26 from cow faeces together with 20 from milk) were tested in inulin without showing any action upon it; and of 133 (95 from human, 20 from horse, and 18 from cow faeces) only 3 showed any action on starch, and these were all of human origin.

This failure to ferment starch on the part of such a large number of organisms, coupled with the small number of bacilli of the *B. lactis aerogenes* group isolated, raised the question as to whether starch-fermenting bacteria were usually present in faeces, and suggested the advisability of carrying out one or two experiments from this point of view.

Human faeces. A loopful of human faeces was put into each of two tubes of bile-salt starch broth, which were incubated at 37° C. In each case there was evident production of acid and gas after 24 hours' growth. From these first two tubes sub-cultures were made into a second pair of starch tubes, and after a day or two from the second pair into a third pair of starch tubes. In none of these sub-cultures was there any production of acid or gas. Plates were also made from one of the first pair and from one of the second pair of tubes and three organisms isolated from each set of plates. None of these organisms caused any change in bile-salt starch broth.

Again, a small quantity of human faeces was put into a tube of ordinary broth. Twenty-four hours' incubation at 37° C. produced an excellent growth. A loopful of this mixed growth was put into bile-salt starch broth and the tube placed at 37° C. No change had occurred at the end of 5 days.

Horse faeces were put into bile-salt starch broth at 42° C. After 48 hours' growth a loopful was transferred to a second tube of starch broth. No change took place in either tube.

Cow faeces. A similar experiment was made with cow faeces with exactly the same result.

To further test this question, tubes of starch broth were inoculated as below and incubated at 37° C.

No. 1	
B. lactis aerogenes *B. coli communis* *B. acidi lactici* *B. cloacae*	no fermentation in 4 days.
No. 2	
B. lactis aerogenes	acid and gas in 48 hours.
No. 3	
B. lactis aerogenes Horse faeces	no fermentation in 4 days.
No. 4	
B. lactis aerogenes Cow faeces	? gas; a sub-culture made into starch gave a negative result.

Another experiment was made on similar lines:—

	Tube 1	2	3	4
Inoculated with	*B. lactis aerogenes*	*B. lactis aerogenes* + horse faeces	*B. lactis aerogenes* + cow faeces	*B. lactis aerogenes* + broth culture of human faeces
After 3 days at 37° C.	? any change	acid + gas	acid + gas	? any change
	Sub-cultures made into starch-broth from each tube.			
After 3 days' incubation	acid + gas	? change	? change	acid + gas
	Sub-cultures made into starch-broth from each tube.			
After 3 days' incubation	acid + gas	acid + gas	?	acid + gas

These results justify the conclusion that the fermentation of starch is a test upon which not much reliance can be placed.

Section VII.

Voges and Proskauer's reaction as a test for the presence of B. lactis aerogenes and B. cloacae.

This continued failure to isolate the *B. lactis aerogenes* raised doubt as to its constant presence in faeces, and suggested the possibility of using Voges and Proskauer's reaction as a rapid test for the presence of this organism and of *B. cloacae* in various materials, and of thus gaining an idea of the extent of their distribution in nature.

It became, therefore, necessary first to ascertain the effect of growth in mixtures upon the appearance of this reaction. For this purpose the following experiments were performed.

Two fermentation tubes filled with 2 % glucose-broth were inoculated

with mixtures of organisms as below and incubated at 37° C. for 3 days. Result :—

Tube No. 1	Percentage of gas	$\frac{H}{CO_2}$	Voges and Proskauer's reaction
B. cloacae (Jordan) *B. capsulatus* (Pfeiffer) *B. lactis aerogenes* (Escherich) *B. coli communis* (Escherich) *B. acidi lactici* (Hüppe) *B. neapolitanus* (Emmerich) *B. pneumoniae* (Friedländer) *B. proteus vulgaris*	50	$\frac{1}{2}$	+

Tube No. 2	Percentage of gas	$\frac{H}{CO_2}$	Voges and Proskauer's reaction
B. coli communis *B. acidi lactici* *B. neapolitanus* *B. pneumoniae* *B. proteus vulgaris*	20	$\frac{2}{1}$	–

Again fermentation tubes containing as before 2 % glucose-broth were inoculated as below, and after 48 hours' incubation at 37° C. yielded as results :—

	$\frac{H}{CO_2}$	Voges and Proskauer's reaction
No. 1		
B. capsulatus *B. coli communis* *B. acidi lactici* *B. neapolitanus* *B. pneumoniae* (Friedländer) *B. proteus vulgaris*	11/10	? trace after standing 24 hours, + after 48 hours.
No. 2		
B. lactis aerogenes *B. coli communis* etc., etc.	1/1	+ after 24 hours.
No. 3		
B. cloacae *B. coli communis* etc., etc.	6/5	? trace after standing 24 hours, + after 48 hours.
No. 4		
B. capsulatus (Pfeiffer) *B. proteus vulgaris*	2/3	? trace after standing 24 hours, + after 48 hours.
No. 5		
B. cloacae *B. proteus vulgaris*	1/2	+ after 24 hours.

A fresh test was made by inoculating fermentation tubes containing bile-salt cane-sugar broth with rabbit faeces + *B. lactis aerogenes*, rabbit faeces + *B. cloacae*, guinea-pig faeces + *B. lactis aerogenes* and guinea-pig faeces + *B. cloacae*. After 48 hours' incubation at 37° C., the results were :—

	Gas %	$\frac{H}{CO_2}$	Voges and Proskauer's reaction
Rabbit faeces + *B. lactis aerogenes*	40	1/1	+
Rabbit faeces + *B. cloacae*	40	1/1	+
Guinea-pig faeces + *B. lactis aerogenes*	10	1/1	trace
Guinea-pig faeces + *B. cloacae*	15	2/1	+

Another series of glucose-broth tubes was inoculated with small quantities of the faeces of various animals and placed at 37° C. After 3 days' incubation the results of growth were :—

Kind of faeces	Percentage of gas in closed arm of tube	$\frac{H}{CO_2}$	Voges and Proskauer's reaction after 24 hours' standing
Rat	80	1/13	–
Donkey	90	3/1	–
Monkey	30	1/5	trace
Rabbit	50	3/1	trace; increased after 48 hours
Mouse	5	1/0	–
Guinea-pig	8	1/0	–
Goat	1 bubble	1/0	–
Horse	3	1/0	–
Calf	nil		? trace

A similar experiment was made with a broth containing 1 % of cane-sugar instead of glucose. After 5 days' incubation at 37° C. the results were :—

Dog	bubble	1/0	–
Goat	10	3/2	–
Mouse	45	2/3	–
Calf	bubble	1/0	–
Rabbit	30	1/3	–
Monkey	10	2/1	–
Guinea-pig	80	6/1	–
Rat	75	2/3	+
Horse	12	9/1	–

From these results we must conclude that though this test succeeds in artificial mixtures, yet when used with natural mixtures the results are not sufficiently definite to allow us to place absolute reliance upon it as evidence of the presence or absence of the organisms under consideration.

Summary.

(1) The fermentation reactions of certain known organisms have been examined and the lactose-fermenters have been arbitrarily divided into four groups according as they do, or do not, decompose cane-sugar and dulcite, and this grouping has been applied to the organisms isolated in the experiments.

(2) Of human faeces 23 samples have been examined and 241 lactose-fermenting bacilli isolated; of animal faeces 25 samples giving 239 organisms; of milk 4 samples and 40 organisms; and of a mixture of tap-water and faeces 7 samples and 75 bacteria.

(3) Of 480 *coli*-like organisms fermenting lactose isolated from human and animal faeces:—

120 (25 p.c.)	belonged to the	1st group	represented by the	*B. acidi lactici* (Hüppe);
178 (37 p.c.)	„	2nd	„ „	*B. coli communis* (Escherich);
110 (23 p.c.)	„	3rd	„ „	*B. neapolitanus* (Emmerich);
72 (15 p.c.)	„	4th	which contains organisms fermenting cane-sugar but not dulcite;	

or, to put it more succinctly 298 (62 p.c.) had no action on cane-sugar, and 182 (38 p.c.) fermented this sugar, and 288 (60 p.c.) decomposed dulcite, while 192 (40 p.c.) did not.

(4) The *B. acidi lactici* (Hüppe) group in certain circumstances disappeared entirely from the faeces.

(5) In all 625 lactose-fermenting bacilli have been isolated, and of these only 4 have been identified as of the *B. lactis aerogenes* type and 19 as *B. cloacae*.

(6) 18 *B. cloacae* were planted on gelatine and examined from time to time during six weeks when they did not show a trace of liquefaction but there was distinct liquefaction in three months.

(7) The *B. coli communis* (Escherich) has been exposed for 358 days to the influence of unfavourable environment without losing any of its fermentative characteristics, or its power of clotting milk and producing indol.

(8) Voges and Proskauer's "Kalilauge-roth-reaktion" has been looked for in the cultures of a large number of organisms and has only appeared when organisms of the *B. lactis aerogenes* or *B. cloacae* groups were present.

(9) At present there is no means of differentiating the lactose-fermenting organisms of human from those of animal origin; or those of normal dejecta from those found in enteritis.

(10) *B. lactis aerogenes* is not the same organism as the *B. acidi lactici* (Hüppe), and these organisms are not simply non-motile forms of *B. coli communis.*

(11) Contrary to Péré's statement, there are dulcite-fermenting *coli* in the faeces of adults as well as of sucklings.

(12) As the *B. coli communis* group seems to be more clearly associated with faeces than the other groups, further research along these lines would in all probability help to place the value of "*coli*" as an index of pollution upon a sound basis.

(13) As the *B. acidi lactici* (Hüppe) group sometimes disappears so quickly from the faeces, it may not be of truly faecal origin, or may require such special conditions for its continued growth that it may eventually come to afford evidence as to the nearness or remoteness of pollution.

(14) In the report of the bacteriological examination of a drinking water all the characters of the organisms isolated should be given, and these should include at least the fermentation reactions in glucose, lactose, mannite, cane-sugar, and dulcite, the gas amount and gas-ratio, and the occurrence of Voges and Proskauer's reaction.

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AN INVESTIGATION UPON THE BLOOD CHANGES FOLLOWING ANTITYPHOID INOCULATION[1].

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and Lieut. F. M. G. TULLOCH, R.A.M.C.

Introductory.

The following investigation was undertaken at the request of the War Office Committee on Antityphoid Inoculation, to ascertain the nature and degree of the changes that occur in the blood after inoculation, and to determine the extent to which these changes are influenced by differences in the dosage of the vaccine.

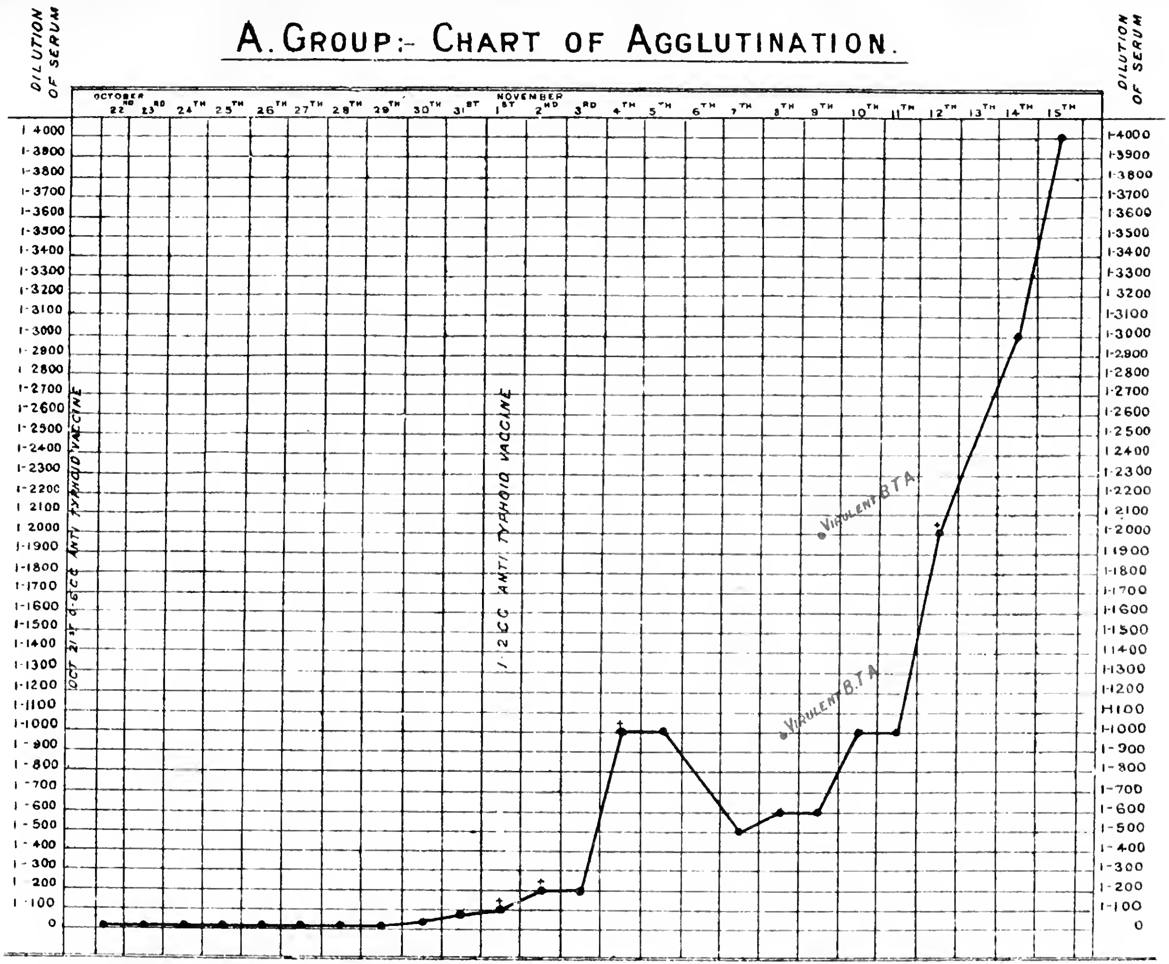
The investigation was carried out at Aldershot upon men of the 2nd Batt. of the Royal Fusiliers who volunteered for inoculation prior to the departure of the regiment for India, where further research upon similar lines will be undertaken by Lieut. Smallman.

The methods by which the vaccine was prepared and standardized and the majority of the technical processes which were employed were those devised by Dr A. E. Wright, late Professor of Pathology at the Army Medical School, Netley. Dr Wright has himself made a large number of observations upon the blood changes following anti-typhoid inoculation but has not had the opportunity of undertaking a systematic estimation of the fluctuations of the protective substances in groups of men. He has, however, urged the advisability of such an investigation, and it is mainly owing to his researches and to his many ingenious methods of blood analysis that such a systematic investigation has now been carried out.

[1] Published with the permission of the Controller of H.M. Stationery Office. This paper will appear simultaneously in the *Journal of the Royal Army Medical Corps*. [Ed.] Tables 1—11 and Charts 1—9 have been supplied to this *Journal* by Messrs Harrison and Sons.

A. Group:- Chart of Agglutination.

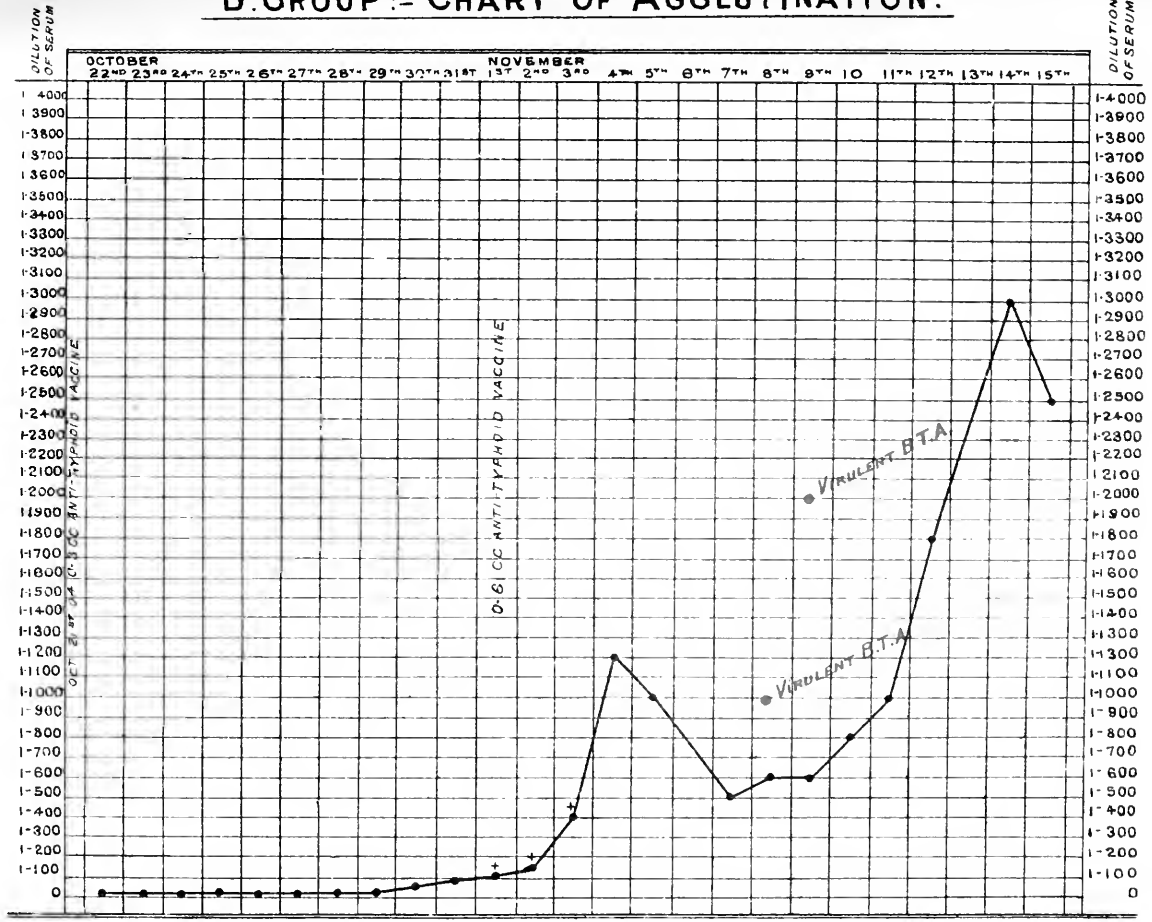
Chart. I.



+ = HIGHEST DILUTION OF THE SERUM TRIED.

B. Group :- Chart of Agglutination.

Chart 2



+ = Highest dilution of the serum tried

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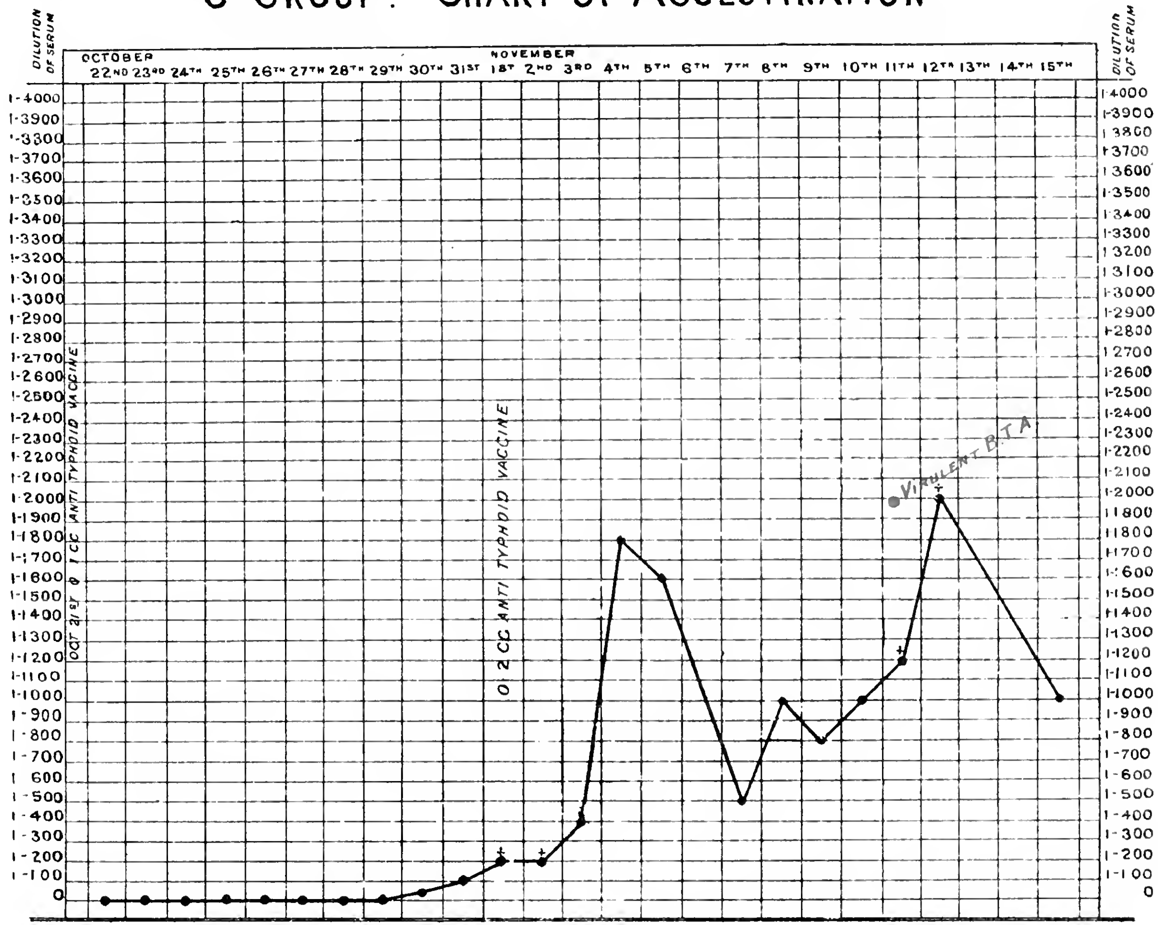
C Group :- Chart of Agglutination

Chart 3



+ = HIGHEST DILUTION OF THE SERUM TRIED

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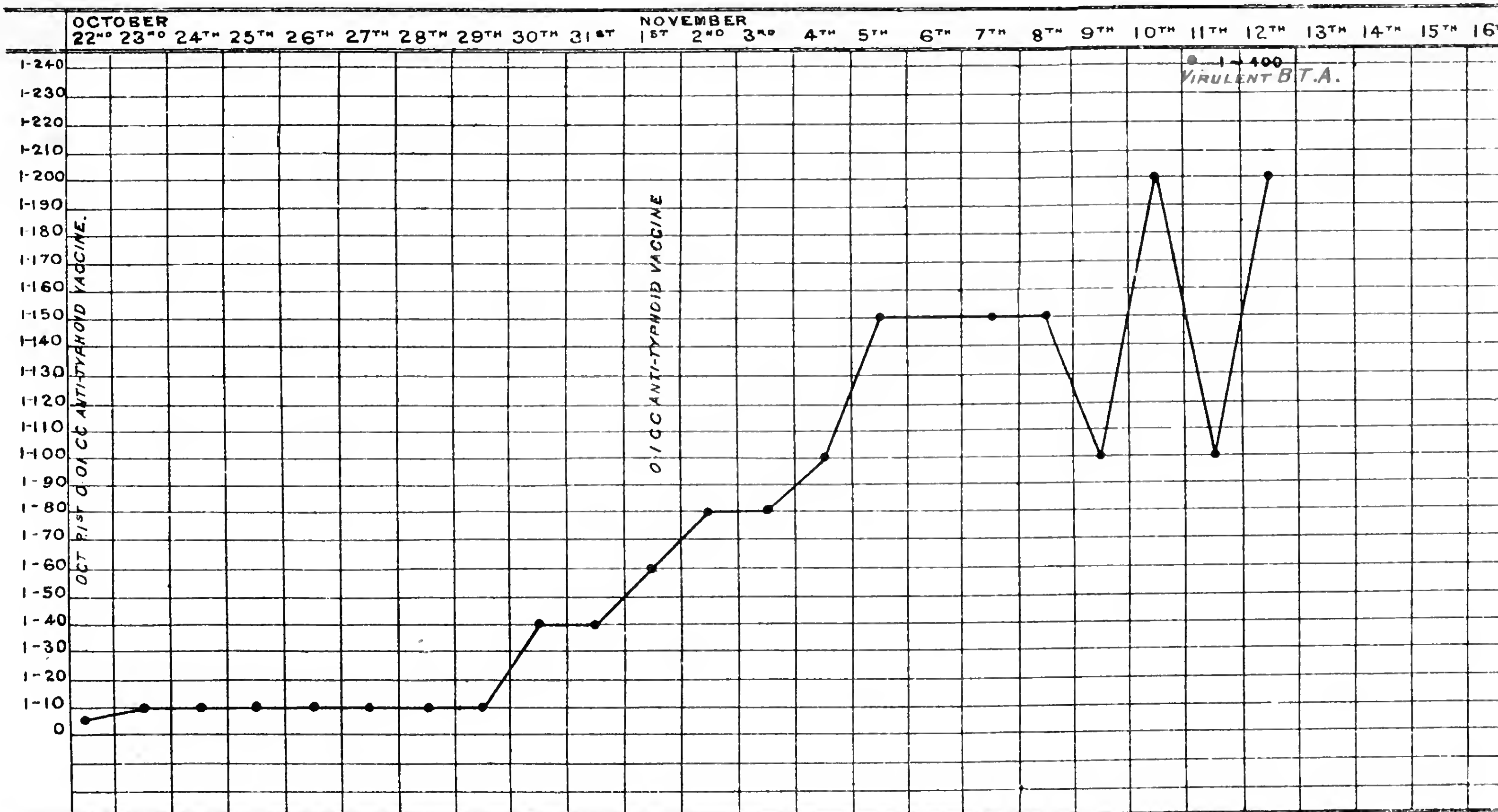
D Group

Chart of Agglutination (on enlarged scale)

Chart 4



+ = HIGHEST DILUTION TRIED

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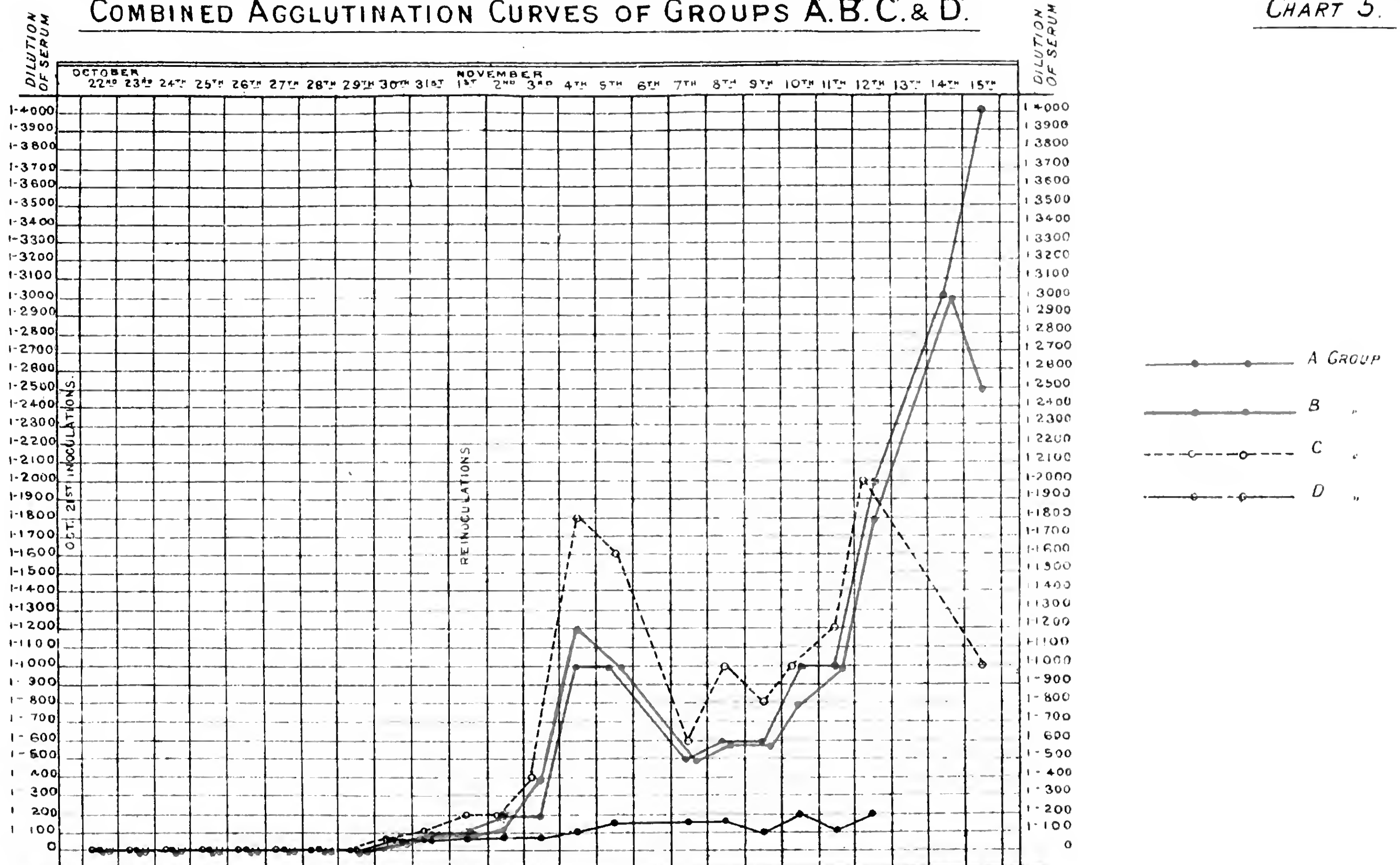
CHART 5.

COMBINED AGGLUTINATION CURVES OF GROUPS "A" "B" "C" & "D".



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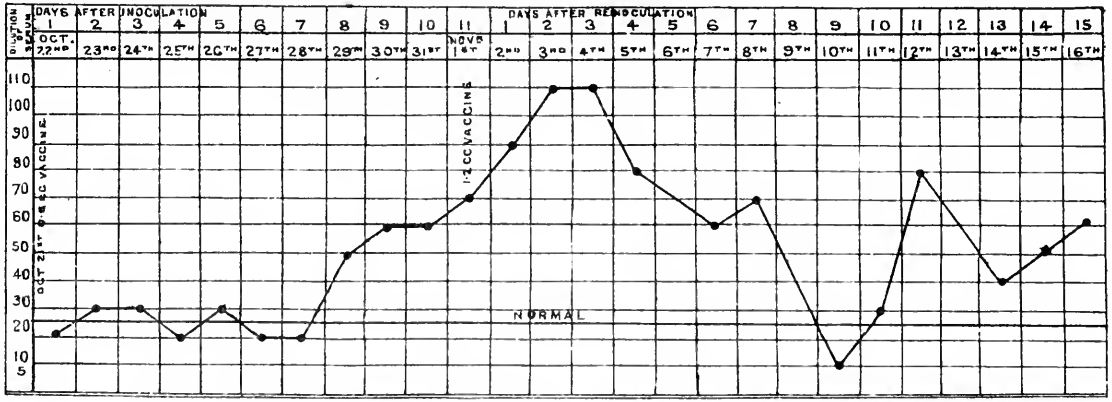
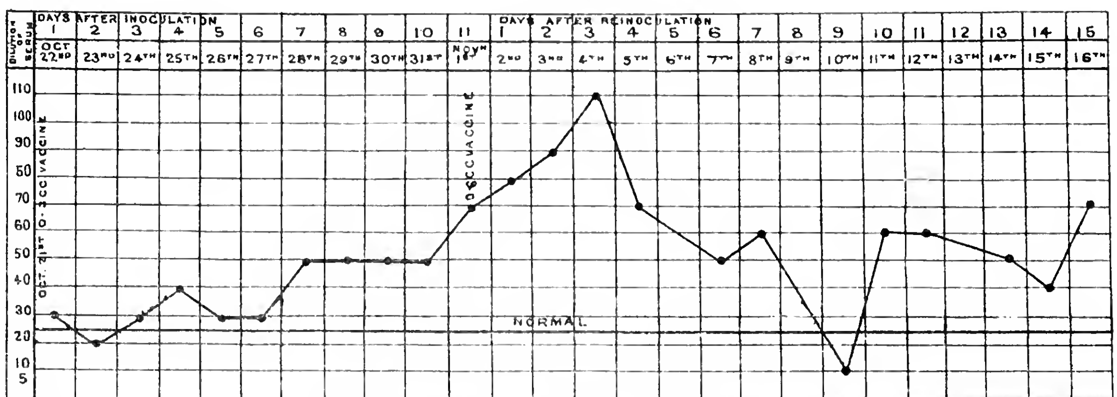
"Bactericidal Substances."

Chart 6

A Group



B Group



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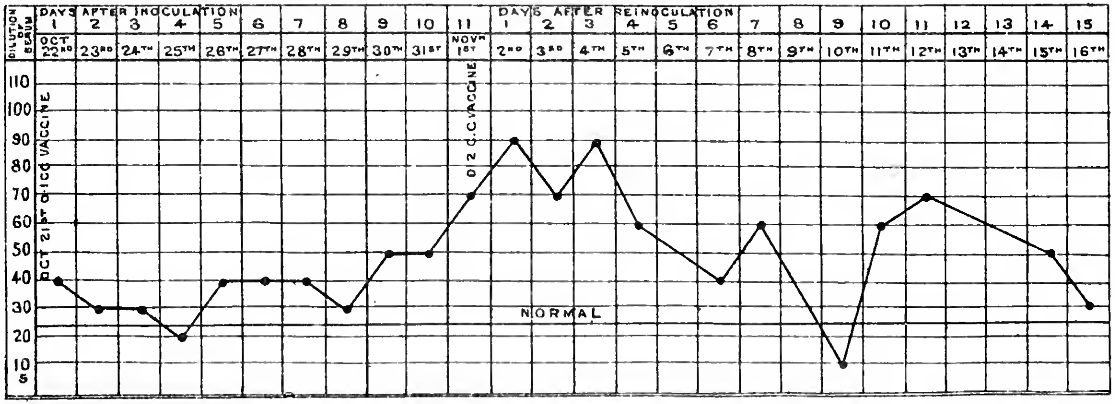
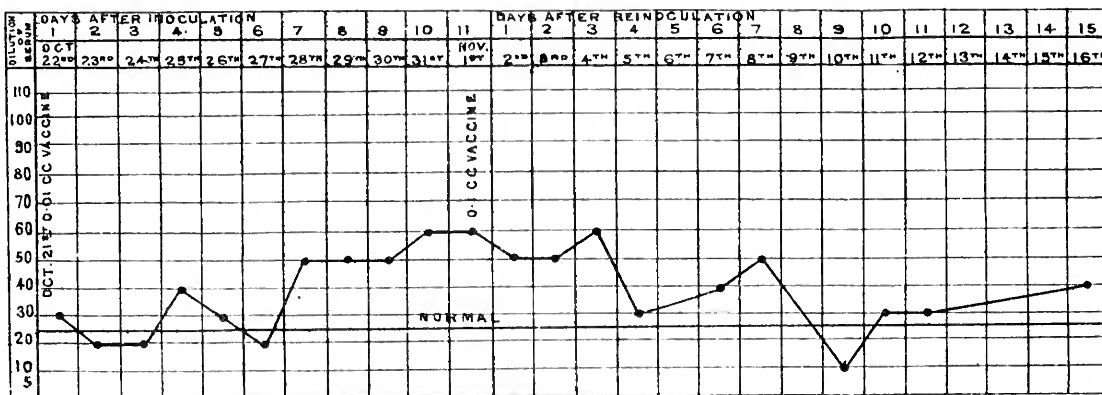
"Bactericidal Substances".

Chart 6
(Continued.)

C Group



D Group



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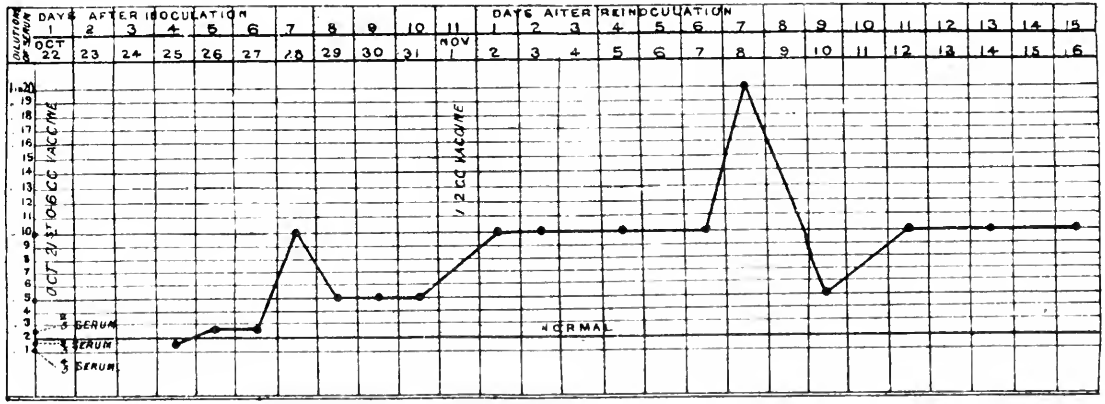
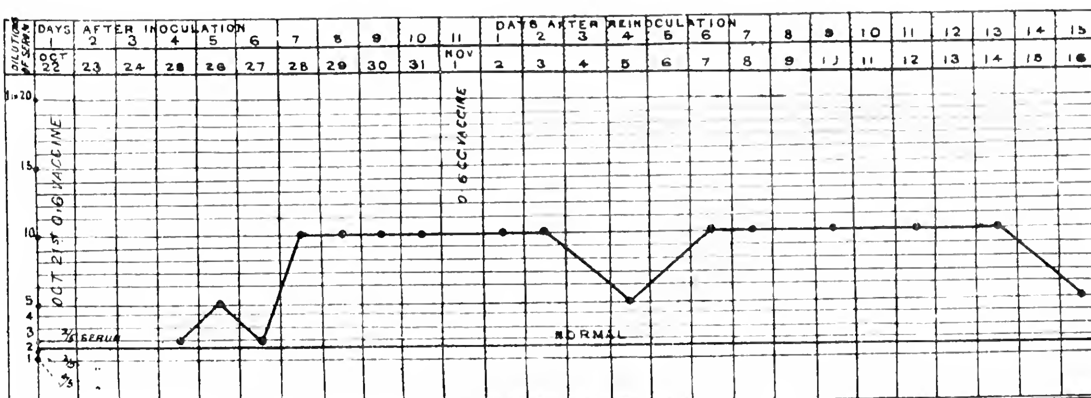
Group "A" "Bacteriolysins" Chart 7



Group "B"



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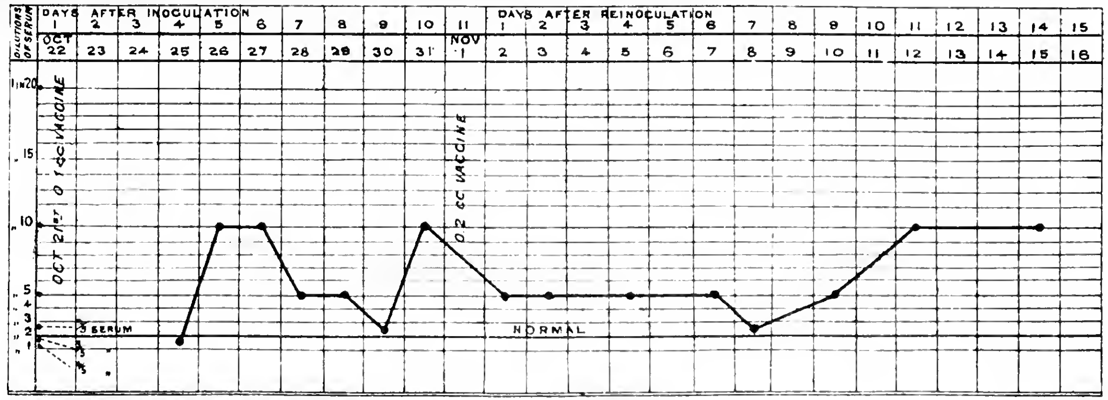
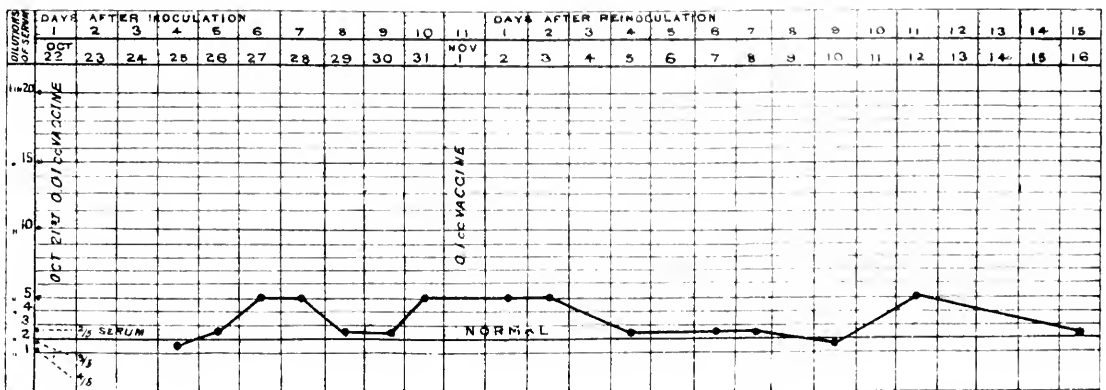
"BACTERIOLYSINS". CHART 7. (CONTINUED)

GROUP "C"



GROUP "D"



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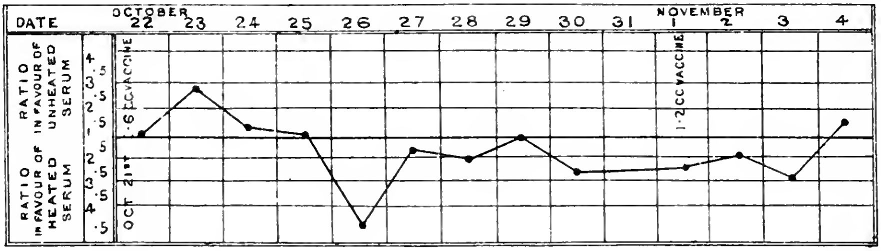
"OPSONINS"

CHART 8

GROUP "A"



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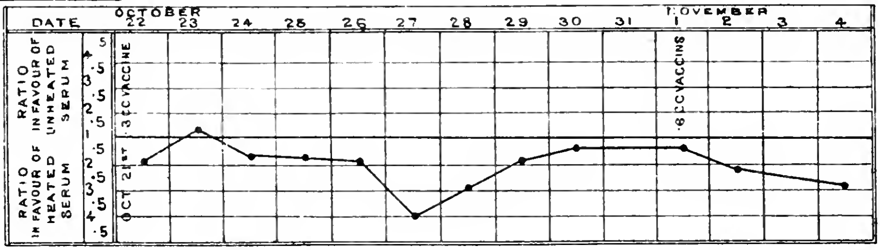

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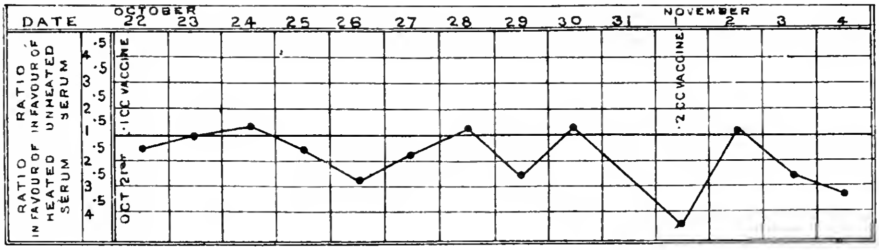

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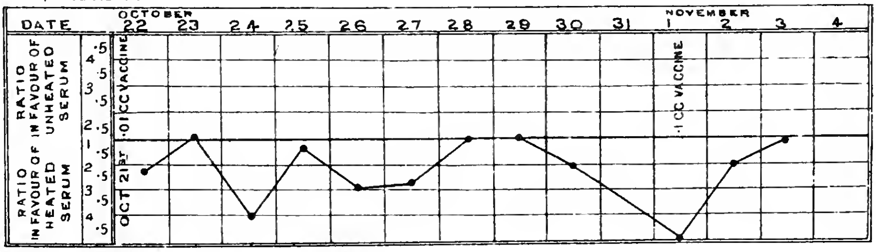

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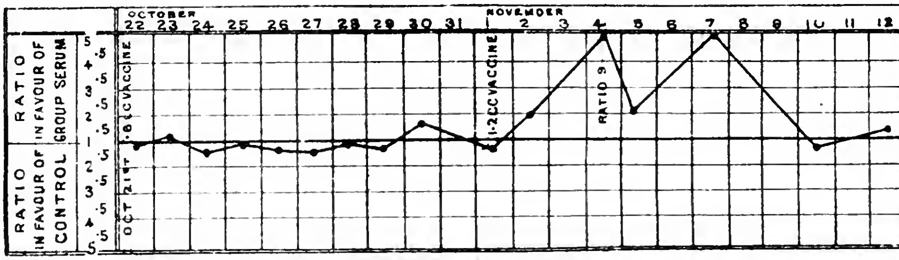
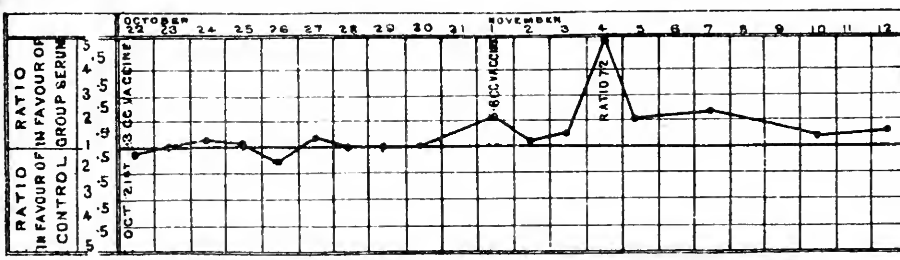
"STIMULINS"

CHART. 9.

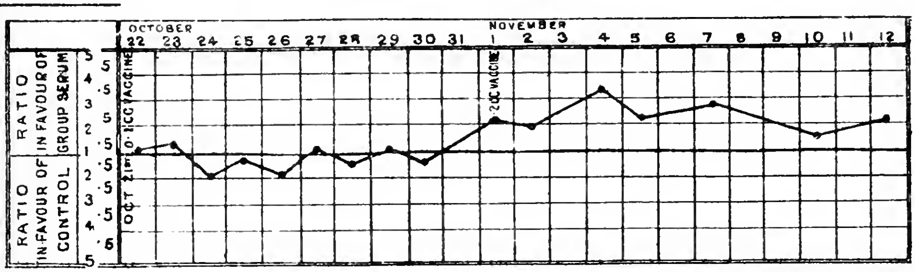
GROUP "A"



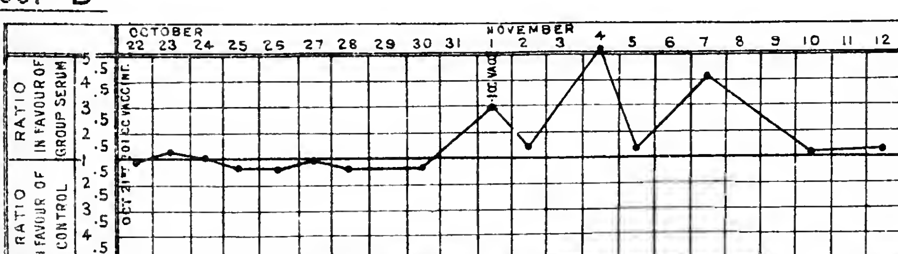
GROUP "B"



GROUP "C"



GROUP "D"



PART I.

Selection and Preparation of the Vaccine.

A. *The strain of B. typhosus selected.*

It was originally intended to employ for this purpose a strain "G," isolated from the spleen at Netley five years ago, which had been largely employed by Dr Wright and one of us (W. B. L.) in the preparation of vaccine and is still employed by Dr Wright for this purpose. In our preliminary work, however, it was found to possess the disadvantage of being a strain which could only be emulsified from agar cultures with great difficulty and at the sacrifice of more time than we were likely to be able to afford. Further experiment resulted in the selection of another strain, "R," of similar origin and of about the same age, which had also been extensively employed at Netley in the preparation of vaccine. This strain was one which furnished a very even and satisfactory emulsion from an agar culture and was thus more suitable for some parts of the work which lay before us. Both strains being of low virulence, preference was accordingly given to that which promised to give more regular results in our test experiments, and the strain "R" was therefore employed both in the preparation of the vaccine and in the daily quantitative tests of the protective substances developed in the blood of the inoculated.

B. *The Vaccine.*

This was prepared on lines similar to those described by Dr Wright and one of us (W. B. L.)[1], with the exception that a young broth culture was employed in place of one 10—14 days old, as was our custom then.

Details of the preparation of the vaccine need not therefore be given except where these differ from the method there described. The culture flasks were incubated for 42 hours at 37° C. After 24 hours the growth was found to be too weak, and as this would have necessitated the inoculation of larger quantities of vaccine than are convenient, the culture was replaced in the incubator for 18 hours. After samples had been drawn for the purposes of enumeration of the bacilli and of retesting the purity

of the growth, the contents of the flasks were mixed in a mixing jar and sterilised in a water-bath at an average temperature of 62° C., maintained for 15 minutes. The temperature was controlled by the use of a second mixing jar, filled with water and fitted up in the same way as that containing the vaccine, but with a thermometer passed through the bung into the centre of the fluid. This jar was previously kept for some hours beside the vaccine jar so that the temperature of each might be identical at the time they were placed in the water-bath. A careful check was kept upon the temperature in the control jar, which during the 15 minutes did not rise above 63·5° C., nor fall below 60° C.

When cool, samples were drawn for testing, and, after proof of sterility by aerobic and anaerobic cultures, ·5 % of lysol was added.

The vaccine was bottled in the manner described in the above-mentioned article.

C. *Standardization of the Vaccine.*

Dr Wright's present method of standardization consists in the employment of a 24-hour broth culture of a known and proved strain of *B. typhosus* and upon an enumeration of the number of bacteria in this culture by the ingenious blood counting method which he has devised [2].

This method we accordingly put into practice with various trial samples of broth and agar vaccines, but although at times we obtained uniform results which were controlled by a 'living count,' made by dilution and plating out on agar, we were unable to obtain consistent satisfactory results, and, in the case of broth cultures, errors of from 50 % to 100 % in counts of the same film, made by different observers, were by no means uncommon. All the devices recommended by Dr Wright, and many others, were employed towards securing a perfect blood film, which in all its parts should represent accurately the relation of the number of germs to the number of red corpuscles, but without giving us any greater confidence in the results obtained. The chief factors which appear to interfere with the accuracy of the method are:

1st. The difficulty in securing a perfect film in which the ratio of germs to cells shall be constant throughout.

2nd. The clumping or agglutination which frequently occurs, especially in broth cultures, leading to great irregularities in the enumeration of a series of microscopic fields.

3rd. The part played by the bacteriolytic action of the blood fluids,

which at times undoubtedly leads to an under-estimation of the number of germs.

Still, at times, the results obtained appeared trustworthy, especially in counting agar emulsions of the strain "R," and in several instances fairly uniform results were obtained from the independent observations of the same emulsion made by three of us and checked by the alternative method of living enumeration which will be detailed below. Some of these agar emulsions, of whose strength we were thus able to be fairly confident, served a useful purpose in the final estimation of the strength of the vaccine employed at Aldershot and will be referred to below.

Counting of the culture by high dilution and the plating out of a measured volume of the dilution on agar depends for its accuracy on the freedom of the broth culture or emulsion from clumps of bacilli, and, further, upon the assumption that the number of dead germs in a young culture of 24–48 hours is small enough to be neglected.

The method described below was employed daily in the standardization of the test-tube cultures used in the analytical work at Aldershot and yielded satisfactory results, but in the measurement of the actual vaccine, grown in flasks on a large scale and mixed, the figures obtained were not to be relied upon, as the microscope showed a considerable quantity of small clumps in the culture, each of which, of course, when inoculated on agar would develop into a single colony and the result be read as 1 germ.

We obtained some assistance, in controlling the figures obtained by the enumeration of the germs in the vaccine, from the chairman of the Antityphoid Committee, Dr C. J. Martin, at whose suggestion estimations were made of the weight of the dried bacterial bodies in a measured quantity of vaccine and a correlation obtained for this weight and the number of bacteria as estimated by the living and dead counting methods. These estimations Dr Martin was good enough to carry out upon a number of trial emulsions and vaccines, and the consistency of the results obtained by him appears to promise that, when such a correlation has once been satisfactorily obtained, this method may be of great help in estimating the strength of a bacterial vaccine.

Dr Martin arrived at his correlation from the results he obtained with two very strong agar emulsions of *B. typhosus* in normal saline solution, these emulsions having been prepared and counted by us carefully, in one instance by both the living and blood methods, in the other by the blood method only, with fairly uniform results. The figures are as follows:

1. *Agar Emulsion "A" of Sept. 30th.*

Counts made at R.A.M. College:

A. *Living method* (by dilution) 32,000 millions per c.c.

B. *Blood method* (by red B. C.)

i. By A. B. S.	26,240 millions	} Av. 30,390	,, ,,
ii. By W. B. L.	34,540 ,,		

Weight Estimation made at Lister Institute by Dr Martin:

i. Residue from 5 c.c.	·0176 gm.	} Dry residue per c.c. ·0035 gm.
ii. ,, ,,	·0175 ,,	

2. *Agar Emulsion "B" of Oct. 4th.*

Counts, Blood method. Made at R.A.M. College:

(1) By A. B. S.	13,040 millions per c.c.	} Av. 9,596 millions per c.c.
(2) ,, W. S. H.	10,000 ,, ,,	
(3) ,, F. M. G. T.	5,750 ,, ,,	

Weight Estimation, made by Dr Martin:

Dry residue per c.c. ·00113 grm.

Translating this last figure into germs from the correlation obtained with agar emulsion "A" gives 10,300 *millions per c.c.* or within 8 °/₀ of the value obtained by taking the average of three independent counting estimations made by different workers using Dr Wright's blood method, viz. 9,596 *millions per c.c.*

Working on the assumption that the correlation obtained from these agar emulsions was accurate, Dr Martin further dried and weighed for us three samples of broth vaccines, "A," "B," and "C," grown and sterilized under identical conditions, with the following results:

"A" *Unlysolised.*

Estimation I.	Residue from 15 c.c.	·0031 gm.	} Av. ·0033 gm.
,, II.	,, ,, ,,	·0034 ,,	
,, III.	,, ,, ,,	·0036 ,,	

"B" *Lysolised.* (·5 °/₀ lysol)

Estimation I.	Residue from 15 c.c.	·0080 gm.	} Av. ·0081 gm.
,, II.	,, ,, ,,	·0082 ,,	
,, III.	,, ,, ,,	·0083 ,,	

"C" 1. *Unlysolised Sample.*

Estimation I.	Residue from 15 c.c.	·0031 gm.	} Av. ·0032 gm.
,, II.	,, ,, ,,	·0033 ,,	

2. *Lysolised Sample.* (·5 °/₀ lysol)

Estimation I. Residue from 15 c.c. ·0078 gm.

The difference which the addition of lysol would make was not anticipated when the estimation of "B" was undertaken, so a control experiment was carried out with two samples of a third vaccine, "C," the first sample unlysolised, the second after the addition of ·5 % lysol. It will be seen from the above figures that the weight of dried bacterial bodies in 15 c.c. of "A" and "C" respectively was practically identical, and, further, from a comparison of the weight of the lysolised samples of "B" and "C" it may be assumed that the weight of bacterial bodies in 15 c.c. of an unlysolised sample of "B" would have been about ·0033 gm.

The weight, then, of dried bacterial bodies in 15 c.c. of each of these three vaccines was as follows:

"A"	·0033 gm.	observed.
"B"	·0033 "	estimated.
"C"	·0032 "	observed.

Putting the correlation figure obtained from the strong agar emulsions into operation this weight would represent approximately 1,700 million bacilli in 1 c.c. of each of these vaccines.

These experiments have been quoted at some length from the important bearing they have upon the standardization of the vaccine employed at Aldershot which was the "B" vaccine of the above series. Vaccine "A" was accidentally contaminated, subsequent to its sterilization and standardization, therefore "B" was prepared in precisely the same manner, and, subsequently, "C," in order to further control the standardization of "A" and "B" and to determine the effect which the addition of lysol had upon the weight of the bacterial sediment.

Careful experiments were made with all three vaccines to determine the number of germs by dilution (living count) and by Dr Wright's blood method, but it was felt that the results obtained were unreliable owing to the presence of numerous clumps of bacilli. The average result of all counts of "A" was 750 millions per c.c. and of "B" 650 millions per c.c., while counts of "C" were quite unreliable.

To test practically the strength of "A" vaccine we had put it to the proof by inoculating ourselves with different doses, three receiving 1 c.c. and the fourth ·1 c.c. The local and general reactions in the case of the 1 c.c. dose were severe and prolonged, lasting for 4–5 days, while with the 0·1 c.c. dose the local reaction was marked and the general reaction moderate, most symptoms disappearing in 48 hours. From these results and from previous experience of the effects of inoculation, as well as from the symptoms described by Dr Wright as following upon a first inoculation of 750–1000 millions bacteria[8], we considered that

the figures which we obtained by counting methods for "A" vaccine considerably under-estimated its strength, and that the estimate arrived at with the help of Dr Martin's correlation, viz. 1700 millions per c.c., afforded a more accurate measure of the number of germs contained in this vaccine "A."

The strength of vaccine "B," which was employed at Aldershot, was therefore taken as being identical with that of "A," and the dosage was fixed on the assumption that it contained 1700 millions of dead typhoid bacilli in 1 c.c.

D. *Dosage.*

The following were the quantities of vaccine "B" employed in the inoculations:

1. For a small group of volunteers to be inoculated with a comparatively large dose.

"A" Group.

1st Inoculation	·66 c.c. = 1133 million bacteria.
2nd "	1·25 c.c. = 2125 " "

2. For the general body of volunteers.

"B" Group.

1st Inoculation	·33 c.c. = 566 million bacteria.
2nd "	·66 c.c. = 1133 " "

3. For a small group of volunteers to be inoculated with a small dose.

"C" Group.

1st Inoculation	·1 c.c. = 170 million bacteria.
2nd "	·2 c.c. = 340 " "

4. In addition to the above, a certain number of men who had been inoculated with typhoid vaccine 5 years previously were persuaded to come forward for re-inoculation, and these were given a very small dose of vaccine with a view to testing the supposed power in such cases of an increased response to inoculation in the elaboration of protective substances. In this group the dosage employed was:

"D" Group.

1st Inoculation	·01 c.c. = 17 million bacteria.
2nd "	·1 c.c. = 170 " "

E. *Number of men inoculated.*

The total strength of the regiment on sailing for India was 5 officers, 358 W. O.'s, N.-C. O.'s and men. 106 volunteered for inoculation and of these 86 subsequently presented themselves for reinoculation.

F. *Selection of groups from among the inoculated for the carrying out of the daily blood tests.*

To secure the regular attendance of these groups the co-operation of the regimental authorities was invoked and sufficient men were induced to volunteer for this purpose from among those inoculated on being excused from morning parade. This plan was found to work satisfactorily, and the men presented themselves daily at the Cambridge Hospital at 7.30 a.m. None had previously suffered from enteric.

G. *Details of the groups.*

"*A*" *group. Large dose.* 6 men, inoculated with ·66 c.c. and re-inoculated with 1·25 c.c. Average age $20\frac{4}{12}$ years. Average service $1\frac{8}{12}$ years.

This group attended regularly until the date of reinoculation when 1 man was dropped out as he was unable to present himself for reinoculation at the same time as the others.

"*B*" *group. Medium dose,* employed for the general body of volunteers. 8 men, inoculated with ·33 c.c. and reinoculated with ·66 c.c. Average age $19\frac{10}{12}$ years. Average service $1\frac{8}{12}$ years.

This group attended regularly and never consisted of less than 7 men.

"*C*" *group. Small dose.* 6 boys, inoculated with ·1 c.c. and re-inoculated with ·2 c.c. Average age $17\frac{1}{12}$ years. Average service $1\frac{10}{12}$ years.

Attended regularly, the smallest number ever present was 4, and it was only on one or two occasions that it fell below 6.

"*D*" *group. Previously inoculated men.* 5 non-commissioned officers, previously inoculated 5 years ago. Inoculated with ·01 c.c. and re-inoculated with ·1 c.c.

Owing to their being often required for duty this group was frequently below full strength, but was never less than 3, except on two occasions when observations were omitted. One N.-C. O. dropped out of the group after re-inoculation, having gone on furlough.

The observations upon this group were concluded a few days prior to the others as these N.-C. O.'s could no longer be spared from their multifarious duties in connection with the departure of the regiment.

H. *The general and local symptoms following the inoculations.*

1*st Inoculations. October* 21*st*, 1904.

"*A*" *group.* (·6 c.c.) General reaction—moderate, in no case severe; local reaction—pain and soreness complained of at the site of inoculation which was marked by redness and swelling.

"*B*" *group.* (·3 c.c.) Symptoms as a whole rather less severe than in "A" group. Local reaction much more marked than the general.

"*C*" *group.* (·1 c.c.) Symptoms moderate except in the case of one boy, aged 15, who was sick and faint in the evening and still unwell next morning with a temperature of 99·8° F.

"*D*" *group.* (·01 c.c.) No appreciable reaction.

The symptoms in all cases disappeared by the end of the second day.

2*nd Inoculations. November* 1*st*, 1904.

"*A*" *group.* (1·2 c.c.) Symptoms, both general and local, as a whole less severe than in "B" and "C" groups. In two cases the men felt absolutely well but for a slight stiffness in the side.

"*B*" *group.* (·6 c.c.) In all cases the local reaction was more severe than after the first inoculation, but the general reaction, with one or two exceptions, was milder.

"*C*" *group.* (·2 c.c.) Both local and general reactions were more marked than after the first inoculations and the symptoms were more severe than those following the reinoculation of "A" and "B" groups. The boy noted above again suffered most.

"*D*" *group.* (·1 c.c.) Slight local reaction only.

In many cases profuse perspiration was complained of on the second night after reinoculation.

PART II.

The Investigation of the Blood Changes following Inoculation.

It had been thought advisable to describe the technique of the various operations with considerable detail for the following reasons:

1st. In order that the value of the results recorded in the protocols and charts may be more accurately assessed.

2nd. That the experiments may be better contrasted with previous work on the same lines.

3rd. That they may be of more service for comparison with any further work that may be done in the direction of improving or modifying the vaccine.

The tests were carried out daily upon the 'pooled' serum of each of the four groups "A," "B," "C," and "D," commencing the day after the first inoculation.

1. *Method of collecting the blood and of 'pooling' the serum of the groups.*

After cleansing and sterilizing the finger a prick was made with a sterile needle and about ·5 c.c. of blood was collected in a sterile capsule. The capsules from each group were placed in separate racks and incubated at 37° C. for 2 hours, after which the serum was separated from the clot by centrifugalisation. The serum from the capsules of each group was then 'pooled' by drawing an equal volume from each capsule into the bulb of a sterile pipette. The pipette was then sealed off at both ends and vigorously shaken to secure a thorough mixture. Each of the four bulbs was then carefully marked in a permanent manner to obviate any chance of subsequent confusion.

In no case was an experiment lost through contamination of the serum collected and pooled in this way.

2. *Preparation of the daily 'stock culture.'*

The stock culture of the "R" strain of *B. typhosus* which was used in the daily estimations was planted out every day at the same hour from the 24 hours' growth in broth inoculated the day before. The same loop was used throughout and no more than the ring of the loop was dipped in the culture—which had been previously well shaken—and used to inoculate the new tube of broth which contained exactly 10 c.c. It was hoped in this way to secure a fairly constant strength of culture for each day's use. In the earlier part of the investigation it did keep fairly constant but, towards the end, the growth became rather more vigorous from the continual daily planting.

3. *Method of diluting and counting of the stock culture.*

This was done daily as it was felt to be of great importance in connection with the measurement of the bactericidal power of the serum.

The method adopted, after much preliminary experimental work, was as follows:

Two series of test-tubes were accurately graduated to contain respectively 9 c.c. and 10 c.c., marks being made on the glass corresponding to these volumes of fluid. These were sterilized and kept ready for use. Two pipettes were employed, one calibrated in the manner devised by Dr Wright [3] to deliver 10 c.mm. of fluid, the other, an ordinary 1 c.c. pipette.

In making the 1/10,000 dilution of the daily culture, employed in the bactericidal estimations, the culture was first vigorously shaken, 1 c.c. was then added by means of a sterile pipette to a test-tube of broth filled up to the 9 c.c. mark; the resulting 1—10 dilution was then in its turn thoroughly mixed and 10 c.mm. transferred by means of the capillary pipette to a test-tube containing 10 c.c. of broth, the pipette being washed out with this broth about 15 times and the whole thoroughly mixed.

This method was employed in place of the diluting pipette recommended by Dr Wright as, in our hands, it gave more accurate results and also effected some saving of time.

The 1—100,000 dilution, used for counting the cultures, was prepared in a similar way by mixing 1 c.c. of the 1—10,000 dilution with 9 c.c. of broth.

The counting was performed by inoculating three agar plates each with 5 c.mm. of the 1—100,000 dilution, the fluid being distributed drop by drop over as large a surface of the plate as possible. As the plates were well dried before use there was no trouble from diffuse growths.

The plates were then incubated at 37° C. for 24 hours and the average number of colonies developed from each of the 5 c.mm. of the 1—100,000 dilution was ascertained and this figure multiplied by 20 millions, the result being taken to represent approximately the number of living bacteria in 1 c.c. of the original culture.

Technique employed in the various quantitative estimations of the protective substances in the serum.

1. *The estimation of the agglutinating power of the sera.*

Wright's 'sedimentation tube' method [4] was employed in the following way. A fresh agar culture of "R" typhoid, of exactly 24 hours' growth, was emulsified every morning in normal saline solution

and approximate uniformity of strength obtained by testing the opacity in a small glass chamber with parallel surfaces against a card with small, clearly printed type. Various dilutions of the pooled serum of each group were made with normal saline solution and equal volumes of these dilutions and of the standardized living emulsion were mixed together, drawn up into a capillary pipette and sealed. The pipettes were set aside at room temperature and the results were read the following morning. Control tubes of diluted emulsion were put up with each series of serum dilutions.

Marked macroscopic clumping, visible to the naked eye, was taken as evidence of a reaction, and uniformity was secured by this duty always being undertaken by the same observer.

2. *The estimation of the bactericidal power of the sera.*

Dilutions of the pooled sera of each group were made in covered sterile watch-glasses, the diluting fluid employed being sterile normal salt solution. The dilutions usually prepared were 1–5; 1 in 10; 1–15; 1 in 20; 1 in 25; 1 in 30; 1 in 35 and 1 in 40; higher dilutions being put up when considered necessary. Equal volumes of each of these dilutions of serum and of the 1 in 10,000 dilution of the broth culture were mixed by means of sterile capillary pipettes, thus giving final dilutions of serum of 1 in 10; 1 in 20; 1 in 30; &c.—the sterile cover of the watch-glass serving as a convenient mixing surface. The mixture was finally drawn up in an unbroken column and the pipette sealed off, care being taken as far as possible to avoid wetting the inside of the pipette above the upper level of the column of fluid.

The pipettes were then incubated at 37° C. for 1 hour, at the end of which time the contents were blown on to the surface of agar plates. In opening the pipettes for this purpose the sealed end was first broken off and the tube tilted to allow the fluid to run towards the upper end of the pipette; a further portion of the free end of the tube was then cut off at a point higher than that which marked the original lower level of the fluid. In this way, when the contents were finally blown out on to the agar they did not pass over a soiled portion of the pipette where, presumably, the serum had not had the same opportunities of acting upon the bacteria as in the mass of the mixture. The plates were incubated at 37°C. for 24 hours and the results as to sterility or otherwise were noted. A control experiment was made each day, using equal

volumes of normal saline and diluted culture in order to get an approximate idea of the number of bacteria with which each dilution of the serum had had to deal.

The experiments were kept as far as possible uniform by using approximately the same volume of diluted serum and diluted culture in each series, and these bactericidal tests were carried out simultaneously by two workers to lessen the chance of differences in the strength of the diluted culture due to further multiplication of the bacteria.

3. *The estimation of the bacteriolytic power of the sera.*

The method described by Dr Wright was adopted for this purpose [2, 5], and the following strengths of sera were employed, the dilutions being made with normal saline solution: undiluted serum, $\frac{4}{5}$, $\frac{3}{5}$, $\frac{2}{5}$, $\frac{1}{5}$, $\frac{1}{10}$, and $\frac{1}{20}$. Equal volumes of each of these dilutions and of a 24-hours' broth culture were mixed, drawn up into capillary pipettes, and incubated for 1 hour at 37° C. The mixtures were then blown out on to slides, dried, fixed with a saturated solution of perchloride of mercury and stained with methylene blue. The specimens were then examined microscopically and the results noted, the following classification being adopted:—

No difference from control	(0)
Some bacteria unaltered, others spherulated	(−)
All bacteria spherulated	(±)
Complete disappearance of all bacteria and spherulated forms	(+)

Further subdivision of the above classes would have been possible had time permitted a more lengthy study of each film, but under the circumstances this was felt to be inpracticable and it was not attempted.

4. *The estimation of the opsonic power of the sera.*

In attempting to measure the amount of opsonin the technique adopted was founded upon Wright and Douglas' modification [6] of the method of quantitatively estimating the phagocytic power of the blood devised by one of us (W. B. L.)[7]. Wright and Douglas showed that in phagocytosis of germs susceptible to the opsonic action of the blood fluids the source of the leucocytes used in the experiment was a matter of subordinate importance, as the phagocytic power of the leucocytes depended, not on any properties inherent to them, but upon the manner in which the germs had been acted upon by the blood fluids.

It should therefore have been possible to measure the opsonic power of the serum of any of the groups by mixing this serum with any freshly washed leucocytes, the result being the same whether the leucocytes used were derived from the blood which furnished the serum or from the blood of any normal individual. Wright and Douglas appear to have demonstrated this fact incontestably in the case of *Staphylococcus pyogenes* and other germs, and as they speak of *B. typhosus* as being also eminently susceptible to the opsonic action of the blood fluids, it was hoped that the method described below would be successful in eliciting any variations which might occur in the opsonic power of the blood during the process of immunisation.

The corpuscles used in the experiments were accordingly taken every morning from one of two normal men, the blood being collected in a sterile capsule and sodium citrate added in the proportion of ·5 %. After centrifugalisation the plasma was pipetted off and the corpuscles were thoroughly washed in three changes of normal saline solution. These washed corpuscles were subsequently used for testing the opsonic power of the various pooled sera. Two capillary tubes were prepared from each group, the first containing the unheated, *i.e.* 'active' serum of the group, the second the same serum 'inactivated' by heating to 60° C. for 15 minutes to destroy whatever opsonins it might contain. The following proportions were adhered to throughout:—

Tube 1. Washed corpuscles. 3 vols.
'Active' serum of group. 3 vols.
Living emulsion of *B. typhosus*. 1 vol.

Tube 2. Washed corpuscles. 3 vols.
'Inactivated' (heated) serum of group. 3 vols.
Living emulsion of *B. typhosus*. 1 vol.

After thorough mixtures these tubes were incubated at 37° C. for 15 minutes, films were then made from them and they were stained and counted in the usual way.

The "phagocytic index," *i.e.*, the average number of bacteria ingested by the polynuclears, was then estimated and the ratio between the phagocytic indices of tubes 1 and 2 was taken as a measure of the opsonic power of the pooled serum of the group.

It may be added that in the majority of these enumerations the nature of the experiment was unknown to the observer, the slides being merely marked with numbers. This system was adopted to eliminate the sub-conscious mental bias which it is so hard to avoid in this kind of

work. Further, in cases in which fewer than 20 polynuclears were counted the result was not recorded.

5. *The estimation of the 'stimulins.'*

Substances which appear to stimulate phagocytosis but differ from opsonins in being thermostable. A short account of the experiments which led to the inclusion in the present investigation of a search for evidence of the development of these stimulins will, it is hoped, shortly be published by one of us (W. B. L.). In these experiments the addition of a small quantity of an immune serum to a normal blood was found to stimulate the phagocytic power of the normal polynuclears towards the particular germ which had been used in immunisation. It was further found that the action of these substances, assuming for them a separate existence, was unaffected by heating to 60° C. for 15 minutes. When Wright and Douglas subsequently published their work on opsonins it was evident from the thermolabile nature of these opsonins that whatever these stimulating substances might be, they were not identical with opsonins.

The method employed to demonstrate their presence was as follows:—

1. A 'control' tube was put up containing

   Normal washed corpuscles. 3 vols.
   Normal heated serum (60° for 15′). 3 vols.
   Emulsion of typhoid (living). 1 vol.

2. The serum of each group was tested against this control in tubes containing the following mixture:

   Normal washed corpuscles. 3 vols.
   Normal heated serum (60° for 15′). 2 vols.
   Pooled heated serum of group (60° for 15′). 1 vol.
   Emulsion of typhoid (living). 1 vol.

In this way all traces of active opsonin were removed, unless some might have adhered to the cells in spite of washing or, if the opsonins be of leucocytic origin, have been freshly secreted. The main difference between the two tubes lay in the replacement of one of the three volumes of heated normal serum in the control, by a corresponding volume of the heated serum of the group. As the experiment progressed this procedure was slightly modified, as will be noted in describing the results obtained.

The system of recording the results was much the same as that adopted in the opsonic investigations and will be detailed later.

PART III.

Records of the Development of the Protective Substances in the Sera of the Inoculated Groups.

As these records are fully detailed in the accompanying protocols and charts it will be unnecessary to add much in the way of commentary; this will therefore be confined to an indication of what appear to be the chief points of interest and to a description of a few of the experiments which were undertaken with a view to the elucidation of some points incidental to the investigation.

Quantitative estimations were carried out daily on the pooled sera of the four groups to determine the development of the following:—

1. Agglutinins.
2. Bactericidal substances.
3. Bacteriolysins.
4. Opsonins.
5. Stimulins.

1. AGGLUTININS.

The charts (Nos. 1–5) fully record, in the form of curves, the history of the development of these substances in the sera of the groups and no protocols are therefore needed to supplement the information thus presented.

The technique described above proved satisfactory and no difficulty was experienced in recording the results. The good emulsifying power of the "R" strain of typhoid simplified this work, and in all cases the control tubes, containing emulsion only, remained evenly turbid and checked the reading of the agglutination tubes.

Normal limits of agglutination.

In the early stages of the experiment the limit of normal agglutination was determined daily in each group. This was found to oscillate between a 1 in 4 and 1 in 10 dilution of serum. Inoculation had no immediate influence upon the amount of agglutinin normally present and no noticeable changes were found until 9 days after inoculation.

First appearance of an increase in the agglutinins.

In all 4 groups the rise commenced 9 days after inoculation and it seems therefore as though dosage had little to do in hastening or retarding their appearance.

A parallel suggests itself between the first rise of the agglutinins after inoculation and the average date of their appearance in the blood in the course of an attack of enteric fever. Though it is a matter of difficulty to fix the latter point with certainty, the two periods evidently correspond closely.

Course of development.

The charts speak for themselves in this respect, but it may be noted that we were somewhat taken by surprise at the rapidity of the rise in the agglutination value and the high levels ultimately attained and on several occasions did not put up high enough dilutions to reach the end point; these occasions are recorded on the charts.

The very high levels attained by the sera of A, B, and C groups will also be noted and the influence of dosage on the levels attained and maintained is unmistakeable.

It was thought well in view of these high readings with a non-virulent strain to ascertain the agglutinating power of the sera upon a virulent strain of *B. typhosus*, and this was kindly sent us from the Lister Institute by Dr Martin. The virulence of this strain had been highly exalted by passage through guinea-pigs, and it was lethal in 24 hours to a 250 gm. guinea-pig in a dose of ·5 c.c. of a 24-hour broth culture. When tested by the same technique against the pooled sera this virulent strain proved even more sensitive to the action of the agglutinins, the readings being higher in every case. The results are recorded separately in each chart.

Following on the rapid initial rise in the agglutinins a fall occurred in all the groups from 4 to 6 days after reinoculation, and, following this, a second rise to a level higher than that previously attained.

This secondary rise commenced in all cases 9 days after reinoculation, and this repetition of a 9-day interval between inoculation and a definite response in the elaboration of fresh agglutinins appears a fact of no little interest.

The very high level reached by group C (over 1—2000) is also remarkable in view of the very small doses of vaccine given, ·1 c.c. and ·2 c.c.

A curious contrast is to be noticed in the effects of the first and second inoculations in A, B, and C groups, the initial rise being greatest in C group and lowest in A, while the opposite is the case in the second rise after reinoculation, the agglutinins here being in direct proportion to the dosage.

2. BACTERICIDAL SUBSTANCES.

The method of measuring these substances described above was adhered to throughout the course of the observations.

Plating out on agar has the advantage over the broth method in that it affords evidence of the *degree* of bactericidal action in serum dilutions too weak to destroy the whole of the bacilli. It was thought also that the detection of contaminating organisms would have been facilitated, but, fortunately, we had hardly any trouble of this sort.

The dilution of culture employed, 1 in 10,000, was, it should be noted, lower than that recommended by Dr Wright, viz. 1 in 100,000. Previous experiment had shown us that the number of germs with the latter dilution of an average broth culture would only average about 20—30 in the volume employed in the tests, and the lower dilution of 1 in 10,000, giving an average number of 200—300 germs, appeared to us to lessen the chance of errors due to large differences in dosage. This must therefore be borne in mind in contrasting our results with those previously recorded inasmuch as the daily task set to the sera in the present experiment was a more severe one.

The two hours' interval which was always allowed between the collection of the blood and the drawing off of the serum from the clot was also fixed as the result of our preliminary work. The time the serum remains in contact with the clot we found to influence the bactericidal power to a very marked degree. Experiments were therefore conducted with a view to determining this point and it was found that the maximum bactericidal effect was obtained in 2 hours, after which period no further rise could be detected by the method employed.

The period which has elapsed since the last meal is also apparently a factor to be reckoned with, but time did not admit of this point being worked out. It seems possible that this may be connected with the polynuclear leucocytosis occurring 2—3 hours after a meal. As the blood of the groups was, however, always collected at the same hour in the morning, after the men's breakfasts, this factor may safely be neglected in the present instance.

Influence of 'pooling' on the bactericidal power.

It was felt that it could not be safely taken for granted that the mixture of equal volumes of the serum of different individuals would prove an accurate method of estimating the average bactericidal power of a group of men. The following experiments were therefore carried out in which the individual values were obtained and the average of the results contrasted with that given by 'pooling' the same sera.

EXPERIMENT I.

A. Serum of A. B. S.	killed in a dilution of	1 in 5.
B. " D. H.	" " "	1 " 5.
C. " F. M. G. T.	" " "	1 " 10.
D. " W. B. L.	" " "	1 " 20.
Pooled sera of *A*, *B*, *C* and *D*	" " "	1 " 10.

EXPERIMENT II.

A. Serum of F. M. G. T.	killed in a dilution of	1 in 20.
B. " D. H.	" " "	1 " 20.
C. " W. B. L.	" " "	1 " 30.
Pooled sera of *A*, *B* and *C*	" " "	1 " 20.

We concluded from these experiments that 'pooling' *does* afford a satisfactory means of estimating the average bactericidal power of a group of men.

Normal limits of bactericidal power, as determined by the above method.

While the values registered for the various groups during the early days of the experiment appear to us to fix the average bactericidal power with a fair degree of accuracy, a number of individual observations were carried out on normal men, before, during, and after the Aldershot work, in order to determine, as far as possible, the limits of normal variation.

In all, 21 separate observations were made, the technique described being adhered to in every case, while the dilutions of serum were the same as those employed in the main experiment.

The results were as follows:

Sterility in 1 in 5	dilution of serum	4 times.
" " 1 " 10	" " "	4 "
" " 1 " 20	" " "	4 "
" " 1 " 30	" " "	3 "
" " 1 " 40	" " "	5 "
" " 1 " 50	" " "	1 time.

There is thus seen to be a wide range of normal variations of bactericidal power, the lowest recorded being 1 in 5 diluted serum, and the highest, on only one occasion, 1 in 50 diluted serum; while the average of these 21 experiments is 1 in 23.

For pooled sera, such as we dealt with, the normal level of bactericidal power, as tested by the above method, may then be taken to lie between the dilutions of 1 in 20 and 1 in 30, and a line has been drawn on the charts to mark this average normal value.

Explanation of the protocols.

The results obtained in each serum dilution are recorded daily, the signs "0" signifying sterility, "+" growth of typhoid on the inoculated plate, and "–" that no experiment was made with that particular dilution.

In addition to recording 'growth' the number of colonies that developed on the plate is recorded in brackets beside each "+" sign, and a careful consideration of these numbers will give a more accurate representation of the bactericidal power of the pooled serum on a given day than is to be obtained from a chart which must necessarily be plotted from an end point, arbitrarily selected.

A record has also been made each day of the results of the 'count' of the broth culture employed, given in millions per c.c.; and in a separate column, headed 'control,' is given the number of colonies which developed on an agar plate from a volume of the 1 in 10,000 dilution of the daily broth culture approximately equal to the volume mixed in each tube with the diluted serum. This figure should be borne in mind in considering the results, as it represents the number of living typhoid germs with which each dilution of serum had to deal.

Explanation of the Charts. (No. 6.)

These are framed from the results recorded in detail in the protocols (Tables 1—4). The line drawn between the serum dilutions of 1—20 and 1—30 represents the average bactericidal power of the normal men whom we tested for this purpose.

In fixing the end point of each daily estimation for the purpose of record in form of a curve we have taken as evidence of a negative bactericidal effect the lowest dilution of serum in which two or more bacilli had survived. A consideration of the protocols will show that in a considerable number of instances a single bacillus has survived, while

in higher dilutions of the same serum the result is either complete sterility or, once more, a solitary survivor of the 200—300 germs introduced. The possibility of these single colonies representing a clump of bacilli formed by the action of agglutination is excluded since they were met with as frequently in estimations of normal blood. In a certain number of instances the solitary colony may have been due to an accidental 'splash' from another tube, made in blowing out the contents on to the agar plate (each plate serving for the testing of three tubes), but, in the majority, it would appear to be due to the fact that in every two or three hundred bacilli there are one or two individuals endowed with a higher power of resistance to the bactericidal action of the serum than their neighbours. That degrees of resistance do occur among the bacilli is evident from the increasing number of survivors the higher the dilution of the serum.

It was thought that the exclusion of these single colonies would accordingly lead to a fairer representation of the actual power of the serum on a given day, and the dilution next below that from which two or more colonies developed was therefore adopted as the end point of sterility and the measure of the bactericidal power. Should this system, however, be considered unjustifiable the necessary corrections of the charts can readily be made from the protocols.

In instances where irregular growths are recorded the cases were judged on their merits, and the system adopted was to ignore an irregular growth provided the higher dilutions gave evidence either of sterility or of the survival of only a single germ.

On one or two occasions the end point was not reached—such observations are of course excluded from the charts, but shown in the protocols.

Commentary.

For six days after the inoculation there was no obvious change in the bactericidal power of any of the groups, and the values recorded are well within the limits of normal variation as determined by the series of individual estimations detailed above. In no case was sterility noted in higher dilutions than 1 in 40 or in lower dilutions than 1 in 20.

The first noticeable rise occurred on the 7th day in B and D groups; A group followed on the 8th day, and C on the 9th.

The subsequent course of the development in each group may be followed on the charts and only special points will be referred to.

The effects of the second dose upon the bactericidal curves.

At the time the reinoculations were performed the bactericidal power was steadily rising in all the groups and it will be seen that in A, B, and C groups this rise was in no degree checked but would rather appear to have been stimulated by the reinoculation.

Whether the rise to the high levels attained during the two or three days following reinoculation is to be attributed to the first or the second dose is a matter for conjecture, but at least it is obvious that no evidence of a negative phase was obtained after reinoculation with doses twice as large as those employed in the first instance.

In the case of group D a fall of one point lasting for two days was observed. As the 2nd dose in this case was 10 times larger than the first, this may possibly indicate inefficient preparation by the very small dose first given, ·01 c.c.

The highest points attained.

In A and B the high level of 1—110 was reached, and in C group 1—90. In D group the figure 1 in 60 was never exceeded.

In all cases these maxima were reached on or before the third day after reinoculation, and on the fourth day, in all the groups, a marked decline commenced, the high levels attained not being approached again. This fall in bactericidal power appeared to be interrupted by a partial recovery on the 7th day after reinoculation, the same period, it may be noted, which elapsed between the primary inoculation and the first rise in bactericidal power.

On the 8th and 9th days after reinoculation a remarkable drop will be noticed in all the curves. As it was not anticipated the end point of sterility was not reached on the 8th day but was found on the 9th day to be 1 in 10 in all the groups.

In our opinion, however, this heavy fall may, at least in part, be accounted for by the fact that, on these two days only, the broth used for the stock culture was different from that which was ordinarily employed. It was noticed at the time to be darker in colour and was only employed owing to an accident to the reserve stock of the usual broth. Though there was none left to test, control experiments were subsequently carried out, using broth of varying degrees of alkalinity to dilute the same serum, and it was found that the reaction of the broth had a powerful effect in modifying the bactericidal power of the serum which

was diluted with it. At the same time, although we were agreed as to this being the possible explanation of the very low values recorded on these two days, it is not impossible that the fall was a true one, and it has therefore been recorded in the curves. It may be noted, however, that the fall is as marked in D group after reinoculation with ·1 c.c. of vaccine as in the other groups with larger doses, while no such fall followed the primary inoculation of group C with a similar dose of ·1 c.c. Whatever the true explanation of the fall it was rapidly recovered from in all the groups.

At the last observations the bactericidal power of groups A and B was still considerably above the normal, standing at 1—60 and 1—70 respectively, while in the case of C and D the values recorded had fallen within the normal limits of variation.

Bactericidal power of the sera tested upon a virulent strain of B. typhosus.

The culture obtained from the Lister Institute was again used for this purpose, and the results of the isolated experiments made are shown in tabular form (Table 5), and may be contrasted with the results obtained on the same day with the non-virulent strain "R."

They have not been entered on the charts, as the control experiments, made with groups of normal sera, showed a lower average value than in the case of non-virulent culture.

In general, the values obtained were decidedly lower than those in the corresponding routine experiments, which would appear to show that a virulent strain of *B. typhosus* is more resistant to the bactericidal action of the serum than the non-virulent strain employed in the inoculations.

Further, it would appear that bacteria of the virulent strain are more uniformly resistant, as the end point of sterility was sharper and there were fewer examples of single germs surviving in stronger dilutions of serum.

3. Bacteriolysins.

The protocols (Tables 6—9) record the daily observations of the bacteriolytic power measured by the technique described above. The curves (Chart 7) have been plotted by taking as an end point the dilution of the serum in which all the bacilli had undergone spherulation and no unaltered rods were detected.

Normal bacteriolytic power.

As in the case of the bactericidal substances this was determined by a series of observations upon the serum of normal individuals and upon the pooled serum of normal men. The technique was of course the same as that used in the daily estimations. The results were very uniform, the end point being reached either with 2/5 or 3/5 diluted serum, no observations showing either a higher or a lower level than this.

A line representing the bacteriolytic power of normal serum has accordingly been drawn on each chart between the serum dilutions 3/5 and 2/5.

Commentary.

A rise in the bacteriolytic power followed inoculation in all the groups, and the degree and persistence of this rise was roughly proportionate to the dose of vaccine employed. The highest level, 1/20, was attained by group A after reinoculation, and in group D no higher value than 1/5, or 1 point above the limits of normal variation, was recorded.

No evidence of a negative phase was manifested after reinoculation.

In group A it is of interest to note that the definite increase of bacteriolysins on the 7th day after inoculation is reproduced 7 days after reinoculation, while in the other groups with smaller doses of vaccine these substances appeared somewhat earlier—on the 5th or 6th day—though they did not subsequently reach such a high level as was attained by group A.

While the difficulties of an accurate classification of these results should be borne in mind, uniformity at least was secured by these estimations being always made and recorded by the same observer. These difficulties were great, and it was regretted that time did not permit of further experiments directed to the elaboration of a more accurate method of measurement.

4. OPSONINS.

Although the results obtained are negative, inasmuch as they fail to record the variations of the opsonins during immunization, they are embodied in the report (Chart 8, Table 10) since they serve to bring out some points of interest.

The results recorded by Wright and Douglas, although few in number, appeared to show that a definite opsonic effect was demonstrable upon typhoid bacilli, but it became evident, after our first observations upon the sera of the groups, that if typhoid opsonins existed their presence could not be demonstrated by the method we adopted. A reference to the protocols and charts will show that the results of the experiments were almost consistently in favour of the heated serum, *i.e.* that phagocytosis was higher in the cases in which the serum had been heated to a temperature sufficient to destroy all active opsonin.

On noting this, a number of observations were made upon the sera of normal individuals and it was found that, although occasionally a positive opsonic effect similar to those recorded by Wright and Douglas was apparent, in the great majority of cases a higher 'phagocytic index' was obtained with 'inactive' heated serum. As the result of numerous experiments directed to the elucidation of this apparently contradictory result, we came to the conclusion that it was mainly accounted for by the bacteriolytic action of the unheated serum upon the typhoid bacilli. In the case of the tubes containing the unheated serum, mixed with the corpuscles and digested at blood heat for 15 minutes, a large proportion of the bacilli were destroyed by the serum and the phagocytes in consequence were provided with fewer opportunities of exercising their function than those in the corresponding tube containing the heated serum, in which bacteriolysis had not occurred to the same extent.

This bacteriolytic action of the serum was noted by Wright and Douglas and pointed out as likely to mask opsonic action in the case of phagocytosis of typhoid bacilli, but, on the other hand, we have found such consistent and active phagocytosis of typhoid bacilli in the case of most normal heated sera,—sera therefore which contained no active opsonin,—that we were unable to convince ourselves of the existence of specific typhoid opsonins.

At all events from our experiments we are unable to endorse the opinion of Wright and Douglas that the typhoid bacillus is 'eminently susceptible' to the opsonic action of the blood fluids.

The daily experiments were however persevered in with the hope first, that an opsonic effect might manifest itself later as immunity became established, secondly, to see whether any correlation could be observed between the degree of bacteriolysis, as recorded in the previous section, and the negative opsonic effects which resulted from our experiments with the pooled sera. As no such evidence of increased opsonic effect

or correlation with the bacteriolysins became manifest the observations were discontinued four days after the reinoculations.

Many attempts were made to obviate the fallacy of bacteriolysis by using heated emulsions, etc., but the results were irregular and unsatisfactory.

We can fully confirm all that Wright and Douglas say as to the alterations which take place in the bacilli, whether inside or outside the cells, in the case of the unheated serum, and the absence of these alterations in the case of the heated serum, but this appears to us simply an evidence of bacteriolysis and to afford no proof of an opsonic action of the serum.

Explanation of the protocols and charts.

The phagocytic index of each tube is recorded and the ratio to 1 has been calculated between the indices of the heated and unheated serum tubes in each experiment. According as this ratio, obtained by dividing the higher index by the lesser, is in favour of the unheated or the heated serum, it is recorded above or below the central line of the chart which is marked "1" and signifies an identity in the indices of the two tubes.

Ex. Heated Serum. Phagocytic index = 20.
Unheated „ „ „ = 10.
Ratio in favour of heated serum = 2.

5. STIMULINS.

Metschnikoff's word 'stimulins' has been provisionally adopted for the theoretical substances dealt with in this section, but further experiment is necessary before their identity can be established. The experiments of Gengou, Klemperer, and Besredka, alluded to by Metschnikoff in his work on Immunity, which attribute to certain normal and immune sera a stimulating action on the phagocytes of the animal into which they are injected, were conducted upon living animals, and it is therefore difficult to compare their results with the experiments of one of us (W. B. L.) already referred to, which were conducted *in vitro* either by his original phagocytic method or by Wright and Douglas' modification. The stimulating effects of normal serum mentioned by the above observers have only been reproduced to a very slight degree by the methods indicated, and the stimulins to which this section refers would appear to be specific in their action and peculiar to immune sera.

Whether identical or not the stimulins of Metschnikoff, like those in question, were thermostable, withstanding the temperature of 60° C. without losing their stimulating properties, and thus in neither case can they be confused with the thermolabile opsonins.

Explanation of the protocols and charts. (Chart 9, Table 11.)

These have been constructed on the same lines as those dealing with the opsonic observations.

The phagocytic index of the control tube being ascertained this was contrasted in each case with the indices of the tubes which contained a trace of the heated pooled serum of each group, the ratio was found and recorded as being either in favour of the control tube or in favour of the group serum tubes. These ratios were taken to plot the curves recorded in the charts, where it will be noted the values above the central line, 1, represent a positive stimulin effect, while those below the line indicate a higher phagocytic power in the control tube.

At first, when little stimulin effect was anticipated, the volume of group serum added to the two volumes of heated normal serum was undiluted; later, from the 4th November onwards, the volume was diluted 1–5 with normal salt solution to lessen the supposed effect of agglutination. The effect of the addition of this amount of salt solution was carefully tested by control experiments and was found not to influence the results.

Living emulsions were employed except on the 10th February, when a heated culture was used.

Commentary.

The results obtained are sufficiently illustrated in the charts and would seem to indicate an acquirement of stimulating properties by the serum about the 11th day after inoculation. No marked contrasts are to be noted in the curves of the four groups, and the stimulating power of the sera did not appear to bear any relation either to the dose of vaccine employed or to the amount of the agglutinins in the various groups. (This latter fact I have also observed in connection with some of my former stimulin experiments. W. B. L.)

PART IV.

General Commentary.

It should be borne in mind that the duration of the investigation only sufficed to trace the origin and early development of the protective substances and the immediate effects of the 1st and 2nd inoculations with different doses of vaccine. The further investigation of these substances in the serum of the various groups is to be continued by one of us (A. B. S.) in India.

1. *General result of the inoculations.*

It will be seen that, even with very small doses of vaccine, a remarkable development of protective substances occurred in the blood of the inoculated. At the conclusion of the investigation, four weeks after the first inoculations, the amount of these substances, in the majority of instances, remained considerably above the normal.

2. *The local and general reactions following on inoculation.*

In no case, even with the highest doses employed, did the reaction appear excessive. At the end of 48 hours all symptoms had disappeared except in the case of a few individuals, in whom pain and tenderness at the site of inoculation persisted for a day or two longer. In general, the reactions were proportionate to the dosage employed.

A contrast was obtained in the case of groups A and B as to the effect of the same dose of vaccine—·6 c.c.—employed in the case of A as a first dose, in the case of B as a second dose following a first inoculation of ·3 c.c. of vaccine. No marked difference was noticed in the reaction in these two instances.

3. *Effects of dosage upon the development of protective substances.*

The advantage appears distinctly to rest with group A, that which received the largest dose, and in the other groups the quantity of those substances developed bears a general relation to the quantity of vaccine employed.

This general relationship of protective substances to dosage of vaccine does not, however, appear to be in proportion to the differences

in dosage; for instance, the values in group B were only slightly lower than those recorded in group A although A received twice as much vaccine as B, and, again, the quantity of protective substances developed in group C, which received but one-sixth of the dose given to A, was remarkably high considering the small dose employed.

The result of the further investigations upon the blood of these groups in India must be awaited before drawing conclusions from the persistence of these protective substances in the blood as to the probable measure of protection afforded by the different doses.

4. *Question of the development of a 'negative phase.'*

No evidence of the development of such a phase was found in any of the groups, either on first inoculation or reinoculation. The system adopted of 'pooling' the serum of the groups does not of course exclude the possibility of such a phase having developed in individual instances, but had such been marked or common our experiments should have given evidence of it, especially in the case of group A. The further possibility of the negative phase being of a very transient character and thus escaping observation is theoretically possible, but it should be remembered that, in all cases, the blood was first tested within 16 hours after inoculation.

It seems probable, therefore, that, with dosage such as we employed, a negative phase if developed is so slight or so transient in nature as to be negligible.

5. *Interspacing of the inoculations and reinoculations.*

The interval selected of 11 days between first and second inoculations appears to be very suitable. At this time the protective substances formed in response to the first inoculation had made their appearance and were rapidly increasing while reinoculation appears to have stimulated rather than retarded their further elaboration.

6. *Results of inoculation in group D.*

This group of older men, previously inoculated with typhoid vaccine 5 years ago, failed to show any marked response to reinoculation with ·01 c.c. of vaccine. They received, therefore, a tenfold dose of ·1 c.c. as

a second inoculation, but here too, as far as the experiment went, no unusual development of protective substances occurred.

Probably the interval of 5 years which in this case had elapsed since the former inoculation was too great.

7. *Value of the agglutinin curve as a measure of the protective substances in general.*

The amount of agglutinins developed in the various groups appears to afford a fair general indication as to the development of the other protective substances, a fact which might perhaps be taken advantage of in future investigations in which it might not be practicable to carry out the more delicate technical processes.

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TABLE 1

A Group.—Bactericidal action of the pooled sera of 6 men, who received a dose of ·6 c.c. Anti-typhoid Vaccine on October 21st, and 1·2 c.c. on November 1st. Varying dilutions of the pooled sera mixed with equal volumes of 1–10,000 diluted 24-hour broth culture of Bacillus Typhosus (R.), incubated for 1 hour at 37° C., and then blown out on to Agar plates.

Date.	Count of culture per c.c.	Serum.													Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	1–80.	1–90.	1–100.	1–110.	1–120.	
Oct. 22nd	586 millions	0	0	0	+	+	+	–	–	–	–	–	–	–	Not counted.
„ 23rd	606 „	0	0	0	+ (1)	+ (3)	+ (5)	+ (29)	–	–	–	–	–	–	About 200 colonies.
„ 24th	486 „	0	+ (1)	0	0	+ (6)	+ (15)	+ (11)	–	–	–	–	–	–	About 150 colonies.
„ 25th	660 „	0	0	0	+ (2)	+ (3)	+ (4)	+ (10)	–	–	–	–	–	–	About 250 colonies.
„ 26th	600 „	–	0	0	0	+ (3)	+ (1)	+ (2)	+ (11)	–	–	–	–	–	About 200 colonies.
„ 27th	746 „	–	0	0	+ (6)	0	+ (1)	+ (4)	+ (1)	–	–	–	–	–	About 300 colonies.
„ 28th	640 „	0	0	0	+ (2)	+ (1)	– (plate dried)	– (plate dried)	–	–	–	–	–	–	About 250 colonies.
„ 29th	600 „	–	0	–	0	0	0	+ (3)	+	+ (15)	+ (47)	+ (60)	–	–	About 200 colonies.
„ 30th	646 „	–	–	–	0	0	0	+ (1)	+ (6)	+ (24)	+ (30)	+ (55)	–	–	About 200 colonies.
„ 31st	750 „	–	–	–	0	0	+ (2)	0	+ (2)	+ (7)	–	–	–	–	About 250 colonies.

Nov.	1st	634 ,,	–	–	–	–	+ (1)	0	0	0	+ (2)	–	–	–	–	About 150 colonies.
,,	2nd	780 ,,	–	–	0	0	0	0	0	0	0	0	–	–	–	About 250 colonies.
,,	3rd	800 ,,	–	–	0	0	0	0	0	0	0	0	+ (1)	0	+ (20)	About 300 colonies.
,,	4th	826 ,,	–	–	–	–	–	–	0	0	0	+ (1)	+ (1)	+ (1)	+ (2)	About 300 colonies.
,,	5th	814 ,,	–	–	–	–	–	–	–	0	0	+ (3)	+ (11)	+ (19)	+ (58)	About 300 colonies.
,,	6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
,,	7th	926 millions	–	–	–	–	–	–	0	+ (3)	+ (9)	+ (16)	+ (42)	+ (57)	+ (about 100)	About 350 colonies.
,,	8th	886 ,,	–	–	–	–	–	0	0	0	+ (12)	+ (9)	+ (34)	–	–	About 350 colonies.
,,	9th	700 ,,	–	–	–	–	–	+ (11)	+ (40)	+ (40)	+ (about 100)	+ (about 100)	+ (about 200)	–	–	About 300 colonies.
,,	10th	616 ,,	–	0	+ (5)	+ (22)	+ (about 50)	+ (about 70)	+ (about 50)	+ (about 70)	–	–	–	–	–	About 250 colonies.
,,	11th	906 ,,	–	0	0	0	+ (2)	+ (1)	–	–	–	–	–	–	–	About 350 colonies.
,,	12th	780 ,,	–	0	0	0	0	+ (1)	0	+ (1)	+ (1)	+ (3)	–	–	–	About 300 colonies.
,,	13th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
,,	14th	960 millions	–	0	0	0	+ (1)	+ (4)	+ (4)	0	+ (20)	+ (18)	+ (about 150)	–	–	About 450 colonies.
,,	15th	704 ,,	–	0	–	+ (2)	0	0	+ (2)	+ (4)	+ (8)	+ (13)	+ (37)	–	–	About 300 colonies.
,,	16th	740 ,,	–	–	–	0	+ (1)	+ (1)	0	+ (4)	+ (10)	+ (17)	–	–	–	About 300 colonies.

+ = Growth ; numbers where given are the number of colonies which grew. 0 = Sterile. – = Not tried.

TABLE 2.

B Group.—Bactericidal action of the pooled sera of 8 men, who received a dose of ·3 c.c. Anti-typhoid Vaccine on October 21st and ·6 c.c. on November 1st. Varying dilutions of the pooled sera mixed with equal volumes of a 1–10,000 diluted 24-hour broth culture of Bacillus Typhosus (R.), incubated for 1 hour at 37° C. and then blown out on to Agar plates.

Date.	Count of culture per c.c.	Serum.													Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	1–80.	1–90.	1–100.	1–110.	1–120.	
Oct. 22nd	586 millions	0	0	0	0	+ (14)	+ (11)	–	–	–	–	–	–	–	Not counted.
„ 23rd	606 „	0	0	0	+ (2)	+ (12)	+ (15)	+	–	–	–	–	–	–	About 200 colonies.
„ 24th	486 „	0	0	0	0	+ (2)	+ (3)	+ (6)	–	–	–	–	–	–	About 150 colonies.
„ 25th	660 „	0	0	0	0	0	+ (3)	+ (15)	–	–	–	–	–	–	About 250 colonies.
„ 26th	600 „	0	0	0	0	+ (2)	+ (2)	+ (10)	–	–	–	–	–	–	About 200 colonies.
„ 27th	746 „	0	0	0	0	+ (2)	+ (1)	+ (1)	–	–	–	–	–	–	About 300 colonies.
„ 28th	640 „	0	0	0	+ (1)	0	0	+ (2)	–	–	–	–	–	–	About 250 colonies.
„ 29th	600 „	–	–	–	0	0	0	+ (5)	+ (5)	+ (7)	+ (15)	+ (25)	–	–	About 200 colonies.
„ 30th	646 „	–	–	–	0	0	0	+ (3)	+ (5)	+ (8)	+ (24)	(45)	–	–	About 200 colonies.
„ 31st	750 „	–	0	–	0	0	0	+ (2)	+ (2)	+ (4)	–	–	–	–	About 250 colonies.

Nov. 1st	634 „	–	–	–	–	0	0	+ (1)	+ (1)	+ (5)	+ (9)	–	–	–	About 150 colonies.
„ 2nd	780 „	–	–	0	0	0	0	0	+ (1)	+ (1)	+ (about 80)	–	–	–	About 250 colonies.
„ 3rd	800 „	–	–	0	0	0	0	0	0	0	+ (1)	+ (4)	+ (2)	+ (about 50)	About 300 colonies.
„ 4th	826 „	–	–	–	–	–	–	0	0	0	0	0	0	+ (2)	About 300 colonies.
„ 5th	814 „	–	–	–	–	–	–	–	0	+ (4)	+ (4)	+ (8)	+ (12)	+ (about 100)	About 300 colonies.
„ 6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
„ 7th	926 millions	–	–	–	–	0	0	+ (2)	+ (12)	+ (18)	+ (25)	+ (21)	+ (58)	–	About 350 colonies.
„ 8th	886 „	–	–	–	0	0	+ (1)	+ (1)	+ (4)	+ (10)	+ (5)	–	–	–	About 350 colonies.
„ 9th	700 „	–	–	–	+ (about 40)	+ (14)	+ (21)	0	+ (about 50)	+ (about 70)	–	–	–	–	About 300 colonies.
„ 10th	616 „	–	0	+ (6)	+ (38)	+ (56)	+ (42)	+ (46)	+ (60)	–	–	–	–	–	About 250 colonies.
„ 11th	906 „	–	0	0	0	0	0	0	–	–	–	–	–	–	About 350 colonies.
„ 12th	780 „	–	0	0	0	0	0	+ (1)	+ (4)	+ (14)	+ (12)	–	–	–	About 300 colonies.
„ 13th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
„ 14th	960 millions	–	0	0	0	0	+ (1)	+ (2)	0	+ (2)	+ (8)	+ (17)	–	–	About 450 colonies.
„ 15th	704 „	–	0	–	0	0	+ (8)	+ (1)	+ (3)	+ (13)	+ (35)	+ (57)	–	–	About 300 colonies.
„ 16th	740 „	–	–	0	0	0	+ (1)	+ (1)	+ (1)	+ (5)	+ (13)	–	–	–	About 300 colonies.

+ = Growth; numbers where given are the number of colonies which grew. 0 = Sterile. – = Not tried.

TABLE 3.

C Group.—Bactericidal action of the pooled sera of 6 boys, who received a dose of ·1 c.c. Anti-typhoid Vaccine on October 21st, and ·2 c.c. on November 1st. Varying dilutions of the pooled sera mixed with equal volumes of a 1–10,000 diluted 24-hour broth culture of Bacillus Typhosus (R.), incubated for 1 hour at 37° C., and then blown out on to Agar plates.

Date.	Count of culture per c.c.	Serum.													Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	1–80.	1–90.	1–100.	1–110.	1–120.	
Oct. 22nd	586 millions	0	0	0	+ (1)	0	+ (2)	–	–	–	–	–	–	–	Not counted.
,, 23rd	606 ,,	0	0	0	0	+ (3)	+ (6)	+ (17)	–	–	–	–	–	–	About 200 colonies.
,, 24th	486 ,,	0	0	0	0	+ (2)	+ (3)	+ (many)	–	–	–	–	–	–	About 150 colonies.
,, 25th	660 ,,	0	0	0	+ (2)	0	0	+ (many)	–	–	–	–	–	–	About 250 colonies.
,, 26th	600 ,,	0	0	0	0	0	+ (4)	0	–	–	–	–	–	–	About 200 colonies.
,, 27th	746 ,,	0	+ (1)	0	0	0	+ (6)	+ (6)	0	–	–	–	–	–	About 300 colonies.
,, 28th	610 ,,	0	0	0	0	0	+ (3)	+ (2)	+ (3)	–	–	–	–	–	About 250 colonies.
,, 29th	600 ,,	–	–	–	–	+ (2)	+ (1)	+ (2)	+ (1)	+ (2)	+ (26)	+ (26)	–	–	About 200 colonies.
,, 30th	646 ,,	–	–	–	+ (1)	0	0	+ (38)	+ (9)	+ (26)	+ (47)	+ (about 100)	–	–	About 200 colonies.
,, 31st	750 ,,	–	–	–	0	0	+ (1)	+ (2)	+ (4)	+ (4)	+ (13)	–	–	–	About 250 colonies.

Nov. 1st	634 ,,	–	–	–	–	+ (1)	0	0	0	+ (8)	+ (8)	–	–	–	About 150 colonies.
,, 2nd	780 ,,	–	–	0	0	0	0	0	+ (1)	0	0	–	–	–	About 250 colonies.
,, 3rd	800 ,,	–	–	0	0	0	0	0	+ (1)	+ (2)	+ (8)	+ (40)	+ (48)	+ (65)	About 300 colonies.
,, 4th	826 ,,	–	–	–	–	–	0	0	0	0	0	+ (5)	+ (3)	+ (3)	About 300 colonies.
,, 5th	814 ,,	–	–	–	–	–	–	0	+ (2)	+ (13)	+ (20)	+ (41)	+ (about 50)	+ (about 70)	About 300 colonies.
,, 6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
,, 7th	926 millions	–	–	–	–	0	+ (2)	+ (1)	+ (2)	+ (19)	+ (36)	+ (67)	–	–	About 350 colonies.
,, 8th	886 ,,	–	0	0	0	0	+ (1)	+ (1)	+ (1)	+ (3)	–	–	–	–	About 350 colonies.
,, 9th	700 ,,	–	–	+ (2)	+ (9)	+ (18)	+ (about 50)	+ (about 70)	+ (about 100)	+ (10)	–	–	–	–	About 300 colonies.
,, 10th	616 ,,	–	0	+ (5)	+ (31)	+ (32)	+ (about 50)	+ (about 60)	+ (about 70)	–	–	–	–	–	About 250 colonies.
,, 11th	906 ,,	–	0	0	0	0	+ (1)	0	–	–	–	–	–	–	About 350 colonies.
,, 12th	780 ,,	–	0	0	0	0	+ (1)	0	0	+ (2)	–	–	–	–	About 300 colonies.
,, 13th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
,, 14th	960 millions	5 members of group absent, experiment omitted.													About 450 colonies.
,, 15th	704 ,,	–	0	0	0	0	0	+ (5)	–	+ (43)	–	+ (about 100)	–	–	About 300 colonies.
,, 16th	740 ,,	–	0	+ (1)	+ (1)	+ (2)	+ (2)	0	+ (6)	+ (1)	–	–	–	–	About 300 colonies.

+ = Growth; numbers where given are the number of colonies which grew. 0 = Sterile. – = Not tried.

TABLE 4.

D Group.—Bactericidal action of the pooled sera of 5 men who had been inoculated against Typhoid Fever 5 years previously, and who received a dose of ·01 c.c. Anti-typhoid Vaccine on October 21st and ·1 c.c. on November 1st. Varying dilutions of the pooled sera mixed with equal volumes of a 1–10,000 diluted 24-hour broth culture of Bacillus Typhosus (R.), incubated for 1 hour at 37° C. and then blown out on to Agar plates.

Date.	Count of culture per c.c.	Serum.													Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	1–80.	1–90.	1–100.	1–110.	1–120.	
Oct. 22nd	586 millions	0	0	0	+ (1)	+ (4)	+ (12)	–	–	–	–	–	–	–	Not counted.
„ 23rd	606 „	0	0	0	+	+	+	+	–	–	–	–	–	–	About 200 colonies.
„ 24th	486 „	0	0	0	+ (2)	+ (15)	+ (6)	+ (23)	–	–	–	–	–	–	About 150 colonies.
„ 25th	660 „	0	0	0	0	0	+ (3)	+ (4)	–	–	–	–	–	–	About 250 colonies.
„ 26th	600 „	0	0	0	0	+ (3)	+ (25)	+ (5)	–	–	–	–	–	–	About 200 colonies.
„ 27th	746 „	0	0	0	+ (4)	+ (2)	+ (3)	+ (2)	+ (11)	–	–	–	–	–	About 300 colonies.
„ 28th	640 „	–	0	0	0	0	0	+ (3)	–	–	–	–	–	–	About 250 colonies.
„ 29th	600 „	–	–	–	0	+ (1)	0	+ (4)	+ (12)	+ (37)	+ (30)	+ (about 150)	–	–	About 200 colonies.
„ 30th	646 „	–	–	–	0	0	0	+ (7)	+ (6)	+ (36)	+ (28)	+ (about 150)	–	–	About 200 colonies.
„ 31st	750 „	–	–	–	–	0	0	0	+ (3)	+ (36)	+ (71)	–	–	–	About 250 colonies.

Nov. 1st	634 ,,	–	–	–	–	0	0	0	+ (8)	+ (20)	+ (36)	–	–	–	About 150 colonies.
,, 2nd	780 ,,	–	–	0	0	0	0	+ (5)	0	+ (about 100)	+ (13)	–	–	–	About 250 colonies.
,, 3rd	800 ,,	–	–	0	0	0	+ (1)	+ (3)	+ (37)	+ (about 50)	+ (37)	+ (about 100)	+ (about 100)	+ about 150)	About 300 colonies.
,, 4th	826 ,,	–	–	–	0	0	0	+ (1)	+ (4)	+ (2)	–	–	–	–	About 300 colonies.
,, 5th	814 ,,	–	–	–	0	+ (5)	+ (31)	+ (about 60)	+ (about 60)	+ (about 100)	–	–	–	–	About 300 colonies.
,, 6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
,, 7th	926 millions	–	–	0	0	0	+ (5)	+ (14)	+ (15)	+ (60)	–	–	–	–	About 350 colonies.
,, 8th	886 ,,	–	–	0	0	0	0	+ (8)	+ (about 80)	+ (about 80)	–	–	–	–	About 350 colonies.
,, 9th	700 ,,	–	–	+ (7)	+ (about 50)	+ (about 100)	+ (about 100)	+ (about 150)	+ (25)	+ (about 200)	–	–	–	–	About 300 colonies.
,, 10th	616 ,,	–	0	+ (8)	+ (35)	+ (9)	–	+ (about 50)	–	–	–	–	–	–	About 250 colonies.
,, 11th	906 ,,	–	0	0	+ (1)	+ (11)	–	+ (25)	–	–	–	–	–	–	About 350 colonies.
,, 12th	780 ,,	–	0	0	0	+ (5)	+ (9)	+ (2)	+ (46)	+ (about 60)	–	–	–	–	About 300 colonies.
,, 13th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
,, 14th	960 millions					4 members of group absent, experiment omitted.									–
,, 15th	704 ,,					3 members of group absent, experiment omitted.									–
,, 16th	740 ,,	–	0	0	0	+ (1)	+ (9)	+ (41)	+ (42)	+ (33)	–	–	–	–	About 300 colonies.

+ = Growth; numbers where given are the number of colonies which grew. 0 = Sterile. – = Not tried.

TABLE 5.

I.—November 8th, 1904.—**Bactericidal action** of A and B Group Sera on a virulent Bacillus Typhosus compared with that on Bacillus Typhosus (R). The virulent culture was one the fatal dose of which for a 250 grm. guinea pig in 24 hours was 0·5 c.c. of a 24-hour broth culture.

	Virulent Bacillus Typhosus.—Count = 480 millions per c.c.						Bacillus Typhosus (R.).—Count = 886 millions per c.c.					
	Serum.					Control.	Serum.					Control.
	1–20.	1–30.	1–40.	1–50.	1–60.		1–20.	1–30.	1–40.	1–50.	1–60.	
A	0	+ (40)	+ (42)	+ (about 200)	+ (about 200)	About 300 colonies.	–	–	–	0	0	About 350 colonies
B	0	+ (51)	+ (about 80)	+ (about 100)	+ (about 200)		–	0	0	+ (1)	+ (1)	

II.—November 9th, 1904.—**Bactericidal action** of A and B Group Sera on the same virulent Bacillus Typhosus, compared with that of the pooled sera of 3 normal men.

Count = 286 millions per c.c.

	Serum.							Control.
	1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	
A	–	0	+ (6)	+ (6)	+ (22)	+ (25)	+ (about 50)	About 200 colonies.
B	–	0	0	+ (1)	+ (2)	+ (13)	+ (about 30)	
Normal	+ (1)	+ (3)	+ (19)	+ (35)	+ (about 50)	+ (about 50)	+ (about 70)	

III.—November 11th, 1904.—**Bactericidal action** of A, B, C and D Group Sera on the same virulent culture, compared with that of the pooled sera of 6 normal men.

Count = 460 millions per c.c.

	Serum.							Control.
	1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	
A	0	+ (1)	+ (7)	+ (about 50)	+ (about 100)	+ (about 150)	–	About 300 colonies.
B	0	0	0	+ (58)	+ (54)	–	+ (about 100)	
C	0	0	0	0	+ (31)	+ (about 150)	–	
D	0	0	+ (2)	+ (50)	+ (50)	+ (about 150)	–	
Normal	0	0	+ (52)	+ (about 70)	+ (about 150)	(+ about 200)	–	

IV.—December 6th, 1904.—**Bactericidal action** of the serum of a normal man on the same virulent culture, compared with that on Bacillus Typhosus (R.).

Culture.	Count.	Serum.								Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	
Bacillus Typhosus (R.) .. .	126 millions ..	0	0	0	0	0	+ (2)	+ (1)	+ (8)	About 150 colonies.
Bacillus Typhosus (virulent) ..	266 „ ..	0	0	0	+ (9)	+ (4)	+ (118)	+ (113)	+ (128)	About 200 colonies.

TABLE 6.

A Group.—Bacteriolytic action of the pooled sera of 6 men who received a dose of ·6 c.c. Anti-typhoid Vaccine on October 21st, and 1·2 c.c. on November 1st. Equal volumes of a 24-hour broth culture of Bacillus Typhosus (R.) and of varying dilutions of the serum were mixed, incubated for 1 hour at 37° C., then blown out on to slides and stained with methylene blue.

Date.	Serum.						
	Undiluted.	4–5.	3–5.	2–5.	1–5.	1–10.	1–20.
*October 22nd	+	–	–	–	–		
* „ 23rd	+	+	+	+	–	–	
„ 24th			Not	tried.			
„ 25th	+	+	±	–	–	–	
„ 26th	+	+	+	±	–	–	
„ 27th			+	±	–	–	0
„ 28th			+	+	+	±	–
„ 29th			+	+	±	–	0
„ 30th			+	+	±	–	–
„ 31st			+	+	±	–	0
November 1st			Not	tried.			
„ 2nd			+	+	±	±	–
„ 3rd			+	+	+	±	–
„ 4th			Not	tried.			
„ 5th			+	+	±	±	–
„ 6th			Not	tried.			
„ 7th			+	±	±	±	–
„ 8th			+	+	±	±	±
„ 9th			Not	tried.			
„ 10th			+	±	±	–	–
„ 11th			Not	tried.			
„ 12th			+	+	+	±	–
„ 13th			Not	tried.			
„ 14th			+	+	+	±	–
„ 15th			Not	tried.			
„ 16th			+	+	±	±	–

+ = Complete bacteriolysis. – = Spherulated and unaltered bacteria present.
± = All bacteria spherulated. 0 = No appreciable bacteriolysis.
* The standard taken for these two days was presence (–) or absence (+) of recognisable bacteria.

TABLE 7.

B Group.—Bacteriolytic action of the pooled sera of 8 men who received a dose of ·3 c.c. Anti-typhoid Vaccine on October 21st, and ·6 c.c. on November 1st. Equal volumes of a 24-hour broth culture of Bacillus Typhosus (R.) and of varying dilutions of the serum were mixed, incubated for 1 hour at 37° C., then blown out on to slides and stained with methylene blue.

Date.	Serum.						
	Undiluted.	4-5.	3-5.	2-5.	1-5.	1-10.	1-20.
*October 22nd	+	+	+	−	−	−	
* „ 23rd	+	+	−	+	−	−	
„ 24th			Not	tried.			
„ 25th	+	+	+	±	−	0	
„ 26th	+	+	+	+	±	−	
„ 27th			+	±	−	−	0
„ 28th			+	±	±	±	−
„ 29th			+	+	+	±	−
„ 30th			+	+	±	±	−
„ 31st			+	±	±	±	−
November 1st			Not	tried.			
„ 2nd			+	+	±	±	−
„ 3rd			+	+	±	±	−
„ 4th			Not	tried.			
„ 5th			±	±	±	−	0
„ 6th			Not	tried.			
„ 7th			+	+	±	±	−
„ 8th			+	+	±	±	−
„ 9th			Not	tried.			
„ 10th			+	+	±	±	−
„ 11th			Not	tried.			
„ 12th			+	+	+	±	−
„ 13th			Not	tried.			
„ 14th			+	+	±	±	−
„ 15th			Not	tried.			
„ 16th			+	±	±	−	0

+ = Complete bacteriolysis. − = Spherulated and unaltered bacteria present.
± = All bacteria spherulated. 0 = No appreciable bacteriolysis.
* The standard taken on these two days was the presence (−) or absence (+) of recognisable bacteria.

TABLE 8.

C Group.—Bacteriolytic action of the pooled sera of 6 "boys" who received a dose of ·1 c.c. Anti-typhoid Vaccine on October 21st, and ·2 c.c. on November 1st. Equal volumes of a 24-hour broth culture of Bacillus Typhosus (R.), and of varying dilutions of the serum were mixed, incubated for 1 hour at 37° C., then blown out on to slides and stained with methylene blue.

Date.	Serum.						
	Undiluted.	4-5.	3-5.	2-5.	1-5.	1-10.	1-20.
*October 22nd	+	+	+	−	−		
* „ 23rd	+	+	+	−	+	−	
„ 24th			Not tried.				
„ 25th	+	+	±	−	0	0	
„ 26th	+	+	+	±	±	±	
„ 27th			+	+	±	±	−
„ 28th			+	+	±	−	0
„ 29th			+	+	±	−	−
„ 30th			+	±	−	0	0
„ 31st			+	+	±	±	−
November 1st			+	+	±	−	−
„ 2nd			+	+	±	−	−
„ 3rd			+	+	±	−	−
„ 4th			Not tried.				
„ 5th			±	±	±	−	0
„ 6th			Not tried.				
„ 7th			+	±	±	−	−
„ 8th			+	±	−	−	−
„ 9th			Not tried.				
„ 10th			±	±	±	−	0
„ 11th			Not tried.				
„ 12th			+	+	±	±	−
„ 13th			Not tried.				
„ 14th			Not tried.				
„ 15th			+	±	±	±	−
„ 16th			Not tried.				

+ = Complete bacteriolysis. − = Spherulated and unaltered bacteria present.
± = All bacteria spherulated. 0 = No appreciable bacteriolysis.
* The standard taken on these days was the presence (−) or the absence (+) of recognisable bacteria.

TABLE 9.

D Group.—Bacteriolytic action of the pooled sera of 5 men who had been inoculated against Typhoid Fever 5 years previously, and who received a dose of ·01 c.c. Anti-typhoid Vaccine on October 21st, and ·1 c.c. on November 1st. Equal volumes of a 24-hour broth culture of Bacillus Typhosus (R.) and of varying dilutions of the serum were mixed, incubated for 1 hour at 37° C., then blown out on to slides and stained with methylene blue.

Date.	Serum.						
	Undiluted.	4–5.	3–5.	2–5.	1–5.	1–10.	1–20.
*October 22nd	+	+	+	−	−		
* „ 23rd	+	+	−	+	+	−	
„ 24th			Not	tried.			
„ 25th	+	+	±	−	−	0	
„ 26th	+	+	+	±	−	−	
„ 27th			+	±	±	−	−
„ 28th			±	±	±	−	0
„ 29th			±	±	−	−	0
„ 30th			±	±	−	0	0
„ 31st			+	±	±	−	0
November 1st			Not	tried.			
„ 2nd			+	+	±	−	0
„ 3rd			+	+	±	−	−
„ 4th			Not	tried.			
„ 5th			±	±	−	−	0
„ 6th			Not	tried.			
„ 7th			±	±	−	−	0
„ 8th			+	±	−	−	0
„ 9th .. .			Not	tried.			
„ 10th			±	−	−	−	0
„ 11th			Not	tried.			
„ 12th			+	+	±	−	0
„ 13th			Not	tried.			
„ 14th			Not	tried.			
„ 15th			Not	tried.			
„ 16th			±	±	−	−	0

+ = Complete bacteriolysis. − = Spherulated and unaltered bacteria present.
± = All bacteria spherulated. 0 = No appreciable bacteriolysis.
* The standard taken on these two days was the presence (−) or the absence (+) of recognisable bacteria.

TABLE 10.

"OPSONINS."

Date.	Group "A."				Group "B."				Group "C."				Group "D."			
	Phagocytic Index		Ratio in favour		Phagocytic Index		Ratio in favour		Phagocytic Index		Ratio in favour		Phagocytic Index		Ratio in favour	
	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.
October 22nd	6·1	5·7	1·06	—	4·1	7·7	—	1·88	5·2	7·2	—	1·38	5·4	11·5	—	2·12
„ 23rd	10·3	3·6	2·95	—	7	5·1	1·37	—	8·9	8·6	1·03	—	10·5	8·5	1·23	—
„ 24th	6·7	5·1	1·3	—	7·4	10·6	—	1·52	7	5	1·4	—	3·7	14·5	—	3·9
„ 25th	5·9	4·8	1·23	—	4·3	7·2	—	1·67	5·2	7	—	1·35	6	8·1	—	1·35
„ 26th	2·1	9·3	—	4·4	5	8·8	—	1·76	4·35	11·8	—	2·66	4·15	12·15	—	2·92

,, 27th	5·64	7·76	—	1·37	3	12	—	4	4·4	7·52	—	1·7	4·32	11·16	—	2·56
,, 28th	4·8	8·6	—	1·8	3·7	10·5	—	2·85	6·2	4·4	1·4	—	3·5	3·4	1·03	—
,, 29th	3·8	3·8	1	1	3·56	6·28	—	1·76	4·4	11·28	—	2·56	4·4	3·96	1·11	—
,, 30th	7·7	17·3	—	2·25	8·15	10·6	—	1·37	10·2	8	1·27	—	8	16·2	—	2·02
November 1st	1·7	3·6	—	2·1	2·2	2·8	—	1·27	7	30·6	—	4·35	4·8	25·3	—	5·25
,, 2nd.. .	2·3	3·5	—	1·52	4·5	9·7	—	2·15	5·4	4·5	1·2	—	3·9	7·2	—	1·85
,, 3rd.. ..	3·76	9·48	—	2·5					5·5	13·4	—	2·42	4·3	4·8	—	1·11
,, 4th.. ..	2·5	1·5	1·66	—	2·5	7·2	—	2·87	2·6	8·2	—	3·15				

TABLE 11.

"STIMULINS."

Date.		Control.	Group "A."			Group "B."			Group "C."			Group "D."		
		Phagocytic Index.	Phagocytic Index.	Ratio in favour of		Phagocytic Index.	Ratio in favour of		Phagocytic Index.	Ratio in favour of		Phagocytic Index.	Ratio in favour of	
				Control.	Group Serum.		Control.	Group Serum.		Control.	Group Serum.		Control.	Group Serum.
October	22nd ..	15	14·6	1·02	—	12·4	1·2	—	16	—	1·06	14·1	1·06	—
"	23rd ..	4	4·5	—	1·13	4·2	—	1·03	5·9	—	1·47	4·9	—	1·23
"	24th ..	15·8	11·6	1·36	—	19·6	—	1·24	9	1·75	—	15·9	1	1
"	25th ..	13·45	12·7	1·05	—	14·7	—	1·09	11	1·21	—	9·3	1·44	—
"	26th ..	21	16·2	1·31	—	13·1	1·63	—	12·5	1·7	—	14·5	1·46	—

,, 27th	..	13·08	9·56	1·37	—	18·04	—	1·38	14·8	—	1·13	12·6	1·03	—
,, 28th	..	15·7	16·1	—	1·01	15·8	—	1·01	10·7	1·46	—	11·6	1·35	—
,, 29th	..	12·92	10·28	1·25	—	13·56	—	1·04	14·8	—	1·14			
,, 30th	..	15·7	24·7	—	1·58	16·5	—	1·05	11	1·43	—	10·8	1·46	—
November 1st	..	5·2	4·1	1·27	—	11	—	2·1	12	—	2·3	15·5	—	2·98
,, 2nd	..	8·5	16·7	—	1·95	9·5	—	1·11	17	—	2	11·3	—	1·33
,, 3rd	..	1·7				2·4	—	1·41						
,, 4th	..	1	9	—	9	7·2	—	7·2	3·3	—	3·3	5·1	—	5·1
,, 5th	..	·6	1·4	—	2·33	1·2	—	2	1·36	—	2·26	·8	—	1·33
,, 7th	..	·65	3·4	—	5·2	1·5	—	2·3	1·8	—	2·76	2·6	—	4
,, 10th	..	2·6	2	1·3	—	3·4	—	1·3	4	—	1·54	2·7	—	1·01
,, 12th	..	2	2·6	—	1·3	3	—	1·5	4·5	—	2·25	2·3	—	1·15

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THE ISOLATION OF B. TYPHOSUS FROM INFECTED WATER, WITH NOTES ON A NEW PROCESS.

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IT has been estimated that 75 % of typhoid epidemics are water-borne, and it is not surprising, therefore, that many attempts have been made to prove the presence of the specific bacillus in the suspected waters, although the bacteriological examination for this purpose has always been recognised as most difficult and uncertain.

The required investigation consists of two distinct processes; first, the isolation of the bacillus, and second, its identification. As regards the process of identification, the application, during the last few years, of the specific reactions of agglutination (Widal-Gruber) and bacteriolysis (Pfeiffer) has rendered the diagnosis reasonably certain, and these two tests together with the morphological and cultural characters now constitute what may be termed a working standard.

For the isolation of the organism, however, there is at present no established method, despite the large amount of attention that has been given to the subject by Continental workers. In the present paper I propose, firstly, to consider very briefly some of the more recent methods that have been advocated for the isolation of the typhoid bacillus; secondly, to enumerate some instances in which the bacillus has been successfully recovered from infected water supplies; thirdly, to describe a precipitation method upon which I have been working, with notes of experiments.

I.

Methods for isolating B. typhosus.

In an infected water the typhoid bacilli are likely to be present in relatively small number, and hence a considerable volume of the water must be examined. It is true that in two or three instances (to be mentioned later) the organism was isolated by direct plating of the water without any preliminary treatment; but the circumstances were exceptional, and as a general rule concentration of the bacterial content of the water must be attempted. The following methods have been used or suggested.

(1) *Filtration.* By passing the water through a bacterial filter, under pressure, all the organisms contained in one or more litres can, in theory, be gathered together in a few c.c. In practice, however, it is certain that a considerable proportion of the organisms is not recovered from the filter. For this and other reasons filtration is being abandoned in the routine bacteriological examination of water for *B. coli*, and therefore cannot be recommended in the more difficult search for *B. typhosus*. (See below, Experiment 9.)

(2) *Chemical precipitation.* The basis of this method is the formation in the water to be examined of an inert precipitate which entangles the bacteria and carries them down.

The following process was devised by a French army surgeon, Vallet[1], and further developed by Schüder[2].

(*a*) *Vallet-Schüder method.* To two litres of the water to be examined are added 20 c.c. of a 7·75 % solution of sodium hyposulphite and 20 c.c. of a 10 % solution of lead nitrate. A precipitate forms, and this is allowed to settle, or is centrifugalized. The clear fluid above is then drawn off and the precipitate dissolved in a saturated solution of the hyposulphite. From this solution plates of suitable media are inoculated.

Schüder states that a water containing 1,366,000 organisms per c.c., after treatment by this method had only 646 per c.c., left.

(*b*) *Ficker's method*[3]. Here the precipitating agent is ferrous sulphate. Two litres of water are rendered faintly alkaline with soda solution and 7 c.c. of a 10 % solution of ferrous sulphate added. The precipitate after

[1] *Archives de Médecine expérim.*, 1901, p. 55.
[2] *Zeitschr. f. Hyg.*, Bd. XLII. 2, pp. 317—326.
[3] *Hygienische Rundschau*, Bd. XIV. No. 1, 1904, pp. 7–9.

settling or being centrifugalized, is dissolved in 25 % solution of neutral potassium tartrate. Ficker claims that 97 to 98 % of the organisms contained in the water are carried down by the precipitate, and that it has no germicidal action on *B. typhosus.*

(*c*) *Alum method.* When alum (double sulphate of aluminium and potassium) is added to tap water, a gelatinous precipitate of aluminium potassium hydrate forms and slowly sinks to the bottom of the vessel. The remarkable power that this precipitate possesses of entangling and carrying down bacteria has been long known. In 1903 I carried out some experiments to test its action on *B. typhosus*[1]. I found that it had practically no germicidal effect on this organism, and it occurred to me that it might be used, therefore, in examining water for the presence of *B. typhosus.* A description of the process, with experimental results, forms the third part of the present paper.

(3) *Serum agglutination.* The use of anti-typhoid serum for the isolation of the bacillus was first suggested by Windelbandt, in a Russian journal. He added 1 c.c. of the infected water to a number of broth tubes, which were then incubated at 37° C. for 3 or 4 days. To the tubes showing a diffused turbidity some drops of an active anti-typhoid serum were added. If clumps formed they were separated out by centrifugalizing, and the clear fluid above drawn off. The deposit was then emulsified in water, and plates prepared from the emulsion. He claims to have thus recovered the bacillus from a broth culture diluted 10 to 30,000,000 times.

Schepilewsky carried out experiments[2] to test Windelbandt's process. The principle remained the same, but he added 10 to 20 c.c. of the infected water to flasks containing 50 c.c. of broth, and used a better medium for plating out. He claims to have recovered the bacillus from tap water inoculated in the following proportions; (i) 1 loop (diam. = 2 mm.) of a typhoid broth culture in 50 litres; (ii) 1 loop (diam. = 1 mm.) of an agar culture in 10,000 litres; (iii) the same in 100,000 litres. (I quote these figures, as I shall comment on them later.)

Altschüller[3] first converted the water under examination into a nutrient medium by adding peptone and salt, then incubated at 37° C., for 24 hours, and drew off quantities of 10 c.c., to which he added the serum. He claims to have isolated the bacillus from 1 litre of water

[1] *Journ. State Medicine*, Sept. 1903.
[2] *Centralbl. f. Bakt.* Orig. xxxiii. No. 5, 1903.
[3] *Centralbl. f. Bakt.* Orig. xxxiii. No. 9.

containing 150 typhoid bacilli and two loopfuls of an agar culture of *B. coli.*

Next for consideration are those methods whose aim is to allow the *B. typhosus* to multiply before attempting isolation, or, as they have been termed,

(4) *Methods of enrichment.* The principle here is the utilisation of a fluid medium which will allow *B. typhosus* to multiply and at the same time prevent, or at least retard, the growth of other organisms, above all of *B. coli.* Various fluids have been devised which more or less inhibit the growth of many water and sewage organisms, but all have proved more favourable to *B. coli* than to *B. typhosus*, and hence in the presence of the former organism have been of little value for the "enrichment" of the latter.

Such was the position when, in 1903, Roth announced[1] that by the addition of caffeine to broth it was possible to check the growth of *B. coli* without interfering much with that of *B. typhosus.* His discovery was fully investigated by Hoffmann and Ficker[2] and applied by them in the examination of faeces and of water for the presence of *B. typhosus.* For the latter purpose they convert the water itself into a nutrient medium by adding 1 % of nutrose, 0·5 % of caffeine, and 0·001 % of crystal violet. The mixture is incubated at 37° C. for not more than 12 or 13 hours. At the end of this time typhoid bacilli will be sufficiently numerous to be isolated on plates without difficulty, whereas colon bacilli will be almost or wholly restrained in their growth. In one experiment they took 1½ litres of water containing 63,000 bacteria per c.c. To this they added 1,500,000 *B. coli* and 2000 *B. typhosi.* The water was treated as just described, and they succeeded in isolating *B. typhosus.* In a second case they recovered *B. typhosus* from water in which it was present in the ratio of 1 to 30,000 of other bacteria.

As to the value of the method of Hoffmann and Ficker there can be no doubt, since it has twice given a successful result in actual practice (see Cases 5 and 6 below); but there is experimental evidence to show that the action of caffeine is by no means uniform.

Kloumann[3] selected three strains of *coli* and typhoid, respectively of different age and origin, and tested the action of caffeine upon them in amounts varying from 0·1 to 1 % of the medium employed. The results showed that on the whole the *coli* strains were more restrained

[1] *Hygienische Rundschau*, XIII. pp. 489—91.
[2] *Hygienische Rundschau*, XIV. pp. 1—7, 1904.
[3] *Centralbl. f. Bakt.* Orig. XXXVI. p. 312, 1904.

in growth than the typhoid, but he concludes by stating that there is no strength of caffeine which will at the same time effectively prevent the growth of *B. coli* and allow the increase of *B. typhosus*; the action of caffeine is relative only, not specific; but although not ideal, the caffeine method is a distinct step forward.

Rietsch[1] tried the effect of caffeine on 22 strains of typhoid and 4 of *coli*. He found that the maximum strength of caffeine which permitted the growth of each strain varied exceedingly, and in the final table of order which he drew up the strains of *coli* did not occupy a detached position.

Courmont and Lacomme[2] find that while caffeine will inhibit *coli*, most strains of typhoid will grow. But typhoid bacilli which have been long isolated and also those which have been recently isolated from blood are very sensitive to its action. They mention a case in which typhoid bacilli isolated from the urine of a patient grew well in caffeine media, but when isolated from the blood of the same patient they would not grow.

I have myself tried the action of caffeine on two strains of typhoid, two of *coli*, and one of *enteritidis* Gärtner. Using broth containing 0·5 % caffeine, I found that Gärtner's bacillus grew very well, the *coli* strains rather feebly, and the typhoid not at all. None of the organisms were of recent origin.

One must conclude, then, that caffeine cannot be entirely relied upon for the elimination of *B. coli* and its allied forms.

One more process must be mentioned, since it is stated to have been frequently used in Paris in the campaign against typhoid fever.

(5) *The process of Cambier*[3]. This author maintains that under given conditions actively motile bacteria will pass more rapidly through a bacterial filter than feebly motile or non-motile bacteria. He first devised a special peptone solution, containing caustic soda, which he found to be favourable to *B. typhosus*, both as regards growth and motility, but unfavourable to *B. coli* and other organisms in both these respects. In a glass vessel containing this peptone solution is placed a small Chamberland filter candle. The interior of the candle is half-filled with the same solution, to this is added 1 c.c. of the fluid under examination, and the whole apparatus incubated at 37° C. Cambier states that under these conditions, if typhoid bacilli are

[1] See *Bull. de l'Institut Pasteur*, Vol. II. p. 597, 1904.

[2] *Journ. de Physiol. et de Pathol. génér.* t. VI. 1904.

[3] *Revue d'Hygiène*, 1902, p. 64.

present, they will, owing to their motility, pass through the filter before colon bacilli or other organisms, and will appear in pure culture in the outer peptone solution. To meet obvious objections, he maintains that while a Chamberland filter does not allow bacteria to pass through at ordinary temperatures, it will do so at 37° C. and under the special conditions mentioned. Cambier's process has been tried by several workers, always with results more or less unfavourable. To give one instance, L. Jacqué[1] found that *coli* grew better than typhoid in the peptone solution, and nearly always appeared first in the outer fluid (1—4 days). From the stools of typhoid patients he generally obtained *B. coli* but never *B. typhosus*, and twice he found in the outer fluid a pure culture of a non-motile coccus.

The truth seems to be that the appearance of bacteria in the external solution is not due to direct transit but to growth through the filter at a favourable temperature, and it is not surprising therefore to find that *B. typhosus* is usually shut out by more hardy forms.

(6) *Solid media.* Whatever process may be used for the preliminary treatment of the water under examination, for the final isolation of the bacillus solid media must be employed. Of these media no description has been given so far, as it seemed more convenient to consider them in one group. Mention must be made of Elsner's and carbolic gelatin, since *B. typhosus* has two or three times been isolated from water on these media. (See Cases 2, 3, 4, below.) Despite these successes, however, for obvious reasons gelatin does not commend itself in the search for typhoid, and during recent years special agar media have been devised, the best known of which are (1) bile-salt agar (MacConkey), (2) alkaline glucose agar (Horrocks), (3) neutral lactose agar, and (4) Drigalski-Conradi agar. All these media aim at the same result, viz., the differentiation of typhoid and *coli* by the naked-eye appearance of their colonies. In this they are all four more or less successful, but by general consent the Drigalski-Conradi medium is the best, and is now nearly always employed in the search for *B. typhosus*. As this is the medium adopted by me in the experiments recorded below I will here state my experience of its use and diagnostic value.

The medium of Drigalski and Conradi may be described as "nutrose-lactose-litmus agar with a trace (0·001 %) of crystal violet." Although it has not fulfilled early expectations in the diagnosis of typhoid fever, it is none the less valuable for water work.

[1] *Centralbl. f. Bakteriol.* Orig. XXXVI. p. 300.

After preparation, it is melted and plates are poured in the ordinary way and allowed to set. Before use they should be dried for a few hours in the warm incubator. They are then inoculated by spreading the suspected fluid over the surface and should be incubated at 42° C.

The growth of many saprophytic organisms is prevented or restrained, but *coli*, typhoid, and allied forms grow readily and produce characteristic colonies. After 24 hours coli colonies are well developed, glistening white by *reflected* light, bright red by *transmitted* light, whereas typhoid colonies are smaller, more delicate in appearance, and bluish-white in colour, never producing any change in the medium. *B. enteritidis* (Gärtner) closely resembles typhoid in its colonies; and certain varieties of *B. coli* which have little or no action on lactose are, in my experience, very difficult to distinguish from typhoid until they have grown for at least 48 hours at 42° C. Then a slight redness in the centre of the colony may be seen, but the growing margin remains blue, and the medium is practically unchanged. Other organisms which I have met with on this medium are *B. fluorescens non-liquefaciens*, *B. pyocyaneus*, streptococci, vibrios, and members of the subtilis and mesentericus groups. (i) The fluorescens and pyocyaneus colonies resemble typhoid, but the extraordinary motility of these bacilli in a hanging-drop preparation generally gives one a clue, and a few subcultures soon settle the diagnosis. (ii) Streptococci are readily distinguished by their very thin, delicate colonies, usually faintly red in colour. (iii) Some vibrio and spirillum forms appear as bluish colonies, but they are not likely to be confused with typhoid. (iv) The subtilis and mesentericus groups present no difficulty. They grow slowly, often not appearing until after 30 hours' incubation, at 42° C., and if the surface of the medium is dry, they do not spread—a point of real value in plate work.

To sum up, this medium is superior to others for separating *coli* and typhoid but is in no sense specific for *B. typhosus*, since several organisms produce upon it colonies of a bluish-white colour which are not easily distinguishable.

Recognizing the limitations of the Drigalski-Conradi medium, further attempts have been made to produce one which shall differentiate *B. typhosus* not only from *B. coli*, but at the same time from closely-allied forms. Among these, the medium devised by Endo, of Tokio[1], has attracted much attention.

Medium of Endo. This is an alkaline lactose agar containing fuchsin, but rendered colourless by the addition of sodium sulphite. Upon it

[1] *Centralbl. f. Bakteriol.* xxxv. 1903, No. 1.

typhoid colonies remain colourless, but *coli* colonies after 20 hours' growth are sufficiently acid to produce a bright red colour.

Petkowitsch[1] instituted a comparison between this medium and that of Drigalski and Conradi. He selected 16 organisms, 6 varieties of so-called paratyphoid bacilli, and 10 varieties of Gärtner's bacillus. He made separate cultures of all these on each medium, and compared the colonies. On the Drigalski-Conradi medium they all produced blue colonies, indistinguishable from each other and from typhoid. On Endo's medium the Gärtner group could be distinguished from the "paratyphoids"; and of these latter three showed slight red coloration, the others, however, resembling typhoid. He considers this medium to be a step forward in the differential diagnosis of *B. typhosus.*

Marschall[2] considers that Endo's medium is in some respects superior to that of Drigalski and Conradi, but Ruata[3] was not favourably impressed, and thinks that it does not rank above other coloured media.

Having now briefly described the principal ways and means for attempting the isolation of *B. typhosus,* I shall next mention the instances I have been able to collect in which the organism has been successfully recovered from infected water-supplies.

II.

According to Lösener, up to 1895 there were 65 cases in which it was claimed that *B. typhosus* had been isolated from water. It is possible that in some of these the bacillus was really that of typhoid, but the evidence of identity is now known to be insufficient and none of these cases can be accepted as conclusive. In the following list only those cases are given in which the diagnosis was confirmed both by agglutination tests and by Pfeiffer's reaction.

(1) Lösener, 1895, from the Berlin water-works. The isolated organism exhibited all the then known characters of *B. typhosus* and was afterwards confirmed as such by Pfeiffer's reaction.

(2) Kübler and Neufeld[4], 1898, from a well on a farm. The bacillus was isolated by direct plating of the water in Elsner's medium, and must have been present in great number. *B. coli* was not found and the well was thought to have been infected by the urine of a typhoid patient.

[1] *Centralbl. f. Bakteriol.* xxxvi. 1904, No. 2.
[2] *Centralbl. f. Bakteriol.* Orig. xxxviii. 1905, No. 3.
[3] *Centralbl. f. Bakteriol.* Orig. xxxvi. 1904, No. 4.
[4] *Zeitschr. f. Hyg.* xxxi.

(3) Fischer and Flatau[1], 1901, from a well in Schleswig-Holstein. The bacillus was isolated on carbol-gelatin plates.

(4) D. Konradi[2], 1902, from a well in Hungary. The bacillus was isolated by direct plating in carbol-gelatin. *B. coli* was not found. Konradi considers that the well was infected by urine.

(5) Jaksch and Rau[3], 1904, at Prague. This case is the most interesting and important of the series. Typhoid fever had been endemic at Prague for years with occasional epidemic outbursts, one of which occurred in the spring of 1904. Jaksch and Rau determined to examine the water for *B. typhosus*. They took a sample of the town supply from a tap in the laboratory, treated it by the caffeine process of Hoffmann and Ficker, and plated out on the Drigalski-Conradi medium. A few red and many blue colonies appeared on the plates. Two of the latter were selected and eventually identified as *B. typhosus*. The bacilli answered to every test, including agglutination and Pfeiffer's reaction, and were highly virulent to guinea-pigs and rabbits.

They next examined the water of the river Moldau, which flows through Prague. In the course of eight days they took five samples of river water and from two they isolated bacilli in every respect identical with those from the tap water, and therefore true typhoid organisms.

(6) Ströszner[4], 1904, from a shallow well near Budapest. He used the process of Hoffmann and Ficker, and for plating out the mediums of Drigalski-Conradi, and of Endo. From both he obtained bacilli which answered to all the standard tests for typhoid. The well had probably been contaminated by urine.

In the following five cases, the isolated organisms reacted positively to agglutination tests, but Pfeiffer's reaction was apparently not applied, so that proof of identity cannot be regarded as complete.

(*a*) Wilson and Wesbrook[5], 1897, from a public water-supply in Minnesota.

(*b*) Hankin[6]. From wells, etc. in India. He used a modification of Parietti's method.

(*c*) Genersich[7], 1899, from public supply at Pécs in Hungary. He made carbol-gelatin plates direct from the water.

(*d*) Tavel[8], 1902, at Olten.

(*e*) Bonhoff[9], 1902, from a well near Marburg.

[1] *Centralbl. f. Bakteriol.* xxix. No. 8.
[2] *Centralbl. f. Bakteriol.* Orig. xxxv. No. 5.
[3] *Centralbl. f. Bakteriol.* Orig. xxxvi. No. 4.
[4] *Centralbl. f. Bakteriol.* Orig. xxxviii. No. 1.
[5] See *Brit. Med. Journ.* Dec. 1897.
[6] *Centralbl. f. Bakteriol.* xxvi. 1899.
[7] *Centralbl. f. Bakteriol.* xxvii. 1899.
[8] *Centralbl. f. Bakteriol.* Orig. xxxiii. p. 166.
[9] *Ibid.* p. 461.

III.

Alum Precipitation Process.

I now proceed to describe the experiments that I have carried out for the isolation of *B. typhosus* from infected water by precipitation with alum. Before coming to details, however, a few words must be said upon the number of typhoid bacilli added to water for experimental purposes. If investigations of this kind are to be of any value some attempt must be made to reproduce numerical conditions that are likely to actually occur in practice. The bacilli must be added to the water in very small amount and the number should be determined.

In describing Schepilewsky's serum process I called attention to the fact that he recovered the typhoid bacillus from water infected in the proportion of 1 loopful (internal diam. = 1 mm.) of an agar typhoid culture to 100,000 litres of water. This statement conveys no real idea of the quantitative value of the process. Using a loop 1 mm. in external diameter, I found that one loopful of an agar typhoid culture contained about 10,000,000 bacilli. Assuming that Schepilewsky in his experiments added the same amount the bacilli were present in the ratio of 1 bacillus to 10 c.c. of water. If it were so, his result was by no means unfavourable, but probably few, even among bacteriologists, would realise that so small a loopful of culture contained so many organisms, as the following instance will show. Schüder's precipitation method was tested experimentally by a bacteriologist in this country, who reported that he had succeeded in recovering *B. typhosus* from 5 litres of water to which one loopful of an agar culture had been added. Assuming that the loop was very small and contained only 5,000,000 bacilli, this would give 1000 bacilli to each c.c. of water, and by plating a few drops on any simple medium the organism could have been at once isolated. The experiment was in fact useless as a test of the value of Schüder's process.

General details of method employed.

A stock solution of alum was first prepared in *distilled* water (10 grm. to 100 c.c.). A known quantity of the infected water was placed in a glass vessel and alum added in the proportion of 0·5 grm. to the litre. As soon as the precipitate had completely formed, the vessel was well shaken to evenly distribute its contents and measured quantities were withdrawn and centrifugalized for 15 minutes, at about 2000

revolutions per minute. The clear water of each centrifugalized tube was then syphoned or poured off without risk of disturbing the small mass of precipitate firmly wedged in the conical extremity, about 0·5 to 1 c.c. of fluid being left behind. In this the precipitate was well stirred, taken up in a sterile pipette, and spread over the surface of one or more Drigalski-Conradi plates. These were incubated at 42° C. for 24 to 48 hours.

Occasionally, after adding the alum the water was allowed to stand in a tall cylindrical vessel until the precipitate had completely settled The clear top water was then syphoned off, leaving about 80 c.c. behind. The precipitate was stirred up in this, centrifugalized and plated out as before.

Notes of Experiments.

(D.-C. = Drigalski-Conradi medium. t. b. = Typhoid bacilli.)

No. 1. (Preliminary) 1·5 litres of tap water were placed in a glass flask. A platinum needle was dipped into a 24 hours' broth culture of typhoid to a depth of 1½ inches, and stirred into the flask. Alum was added and precipitate allowed to form. After shaking, 8 c.c. were withdrawn and centrifugalized, and the sediment spread on D.-C. plates and incubated at 42° C. In 24 hours, plates were covered with typhoid colonies in pure culture. From their number, it was estimated that 300,000 bacilli were introduced into flask by needle. After 2 days, flask was well shaken and 0·1 c.c. plated direct on D.-C. medium. 220 typhoid colonies formed, indicating that flask now contained 3,000,000 t.b. After 9 days, t.b. were not found in 8 c.c. of contents.

Remark. Despite the presence of alum, t.b. multiplied for a day or two.

No. 2. A thin needle was dipped for 1 inch into an old t.b. broth culture and stirred into 10 c.c. of water. Of this, 0·1 c.c. was added to 1 litre of tap water, which was well shaken, and 1 c.c. plated direct on D.-C. No typhoid colonies grew. After precipitation and centrifugalization, t.b. were recovered from the water in the proportion of 1 bacillus to 4 c.c.

No. 3. On same lines as Nos. 1 and 2, but 1/50 of a medium-sized loop of t.b. broth was put into the flask. It was found that 1/50 loop contained 7000 bacilli, which were recovered in abundance by precipitation etc.

No. 4. One loopful of t.b. broth (14 days old) was put into 10 c.c. of sterile water. From this dilution 3 loopfuls were put into 1 litre of tap water, and 3 also into a tube of glucose peptone water. This tube took 72 hours to become acid, so that 3 loopfuls of the dilution evidently contained very few bacilli. Flask of tap water, after inoculation, was treated as usual, and eventually 3 typhoid colonies were obtained from 100 c.c. of water.

No. 5. Into 1 litre of tap water typhoid and colon bacilli were introduced, 1 c.c. was immediately plated on D.-C., and was found to contain 15 typhoid and 4 colon bacilli. After precipitation and centrifugalization the two organisms were recovered

in about the same ratio. Thus, a little of the centrifugalized sediment spread on D.-C. gave 36 typhoid and 9 colon colonies. After 24 hours, flask well shaken and again examined. The organisms were recovered in ratio of 17 typhoid to 18 coli. After 3 days, 1 c.c. from flask contained 3 coli but no typhoid. The latter were evidently rapidly dying out.

No. 6. 1/200 loopful of t.b. broth (14 days old) was added to 1 litre of tap water. T.b. were found to be absent from 1 c.c.

A comparison was instituted between the precipitation method and filtration through a Chamberland filter, 500 c.c. being used for each purpose. The final result was that filtration indicated 1 t.b. in 20 c.c., whereas precipitation indicated 3 t.b. in 20 c.c.

No. 7. To determine percentage of t.b. carried down by the precipitate. 1 litre of sterile tap water was inoculated with typhoid, well shaken, and agar-plates prepared, each containing 0·5 c.c. of the infected water. Alum was then added, and some of the water withdrawn and centrifugalized. From the clear water above the sediment agar-plates were prepared as before, each with 0·5 c.c. Both sets of plates were incubated at 37° C. for 40 hours, and the colonies then counted.

Before precipitation, 1 c.c. of water = 125 bacilli.
After ,, ,, ,, = 16 ,, .

Therefore, 87 % of bacilli were carried down by precipitation.

The satisfactory results obtained thus far led me to test the method on a larger scale, and in the remaining experiments here recorded a galvanized iron tank was used.

No. 8. 130 litres of tap water were run into tank, to which was added 1 litre of sterile water containing 120,000 t.b., and the whole well stirred up. After 24 hours, 250 c.c. were removed from the tank, precipitated, centrifugalized and plated out as usual. Typhoid colonies appeared on the plates in due course, but the number suggested that the bacilli had undergone rapid diminution in the 24 hours following their introduction into the tank. This was confirmed later (see No. 10). After 10 days, tank water was again examined, but no t.b. found in 500 c.c.

No. 9. Tank with 130 litres of tap water to which were added 70,000 t.b. well distributed. After 3 hours, (*a*) 500 c.c. were withdrawn and filtered through a hollow Chamberland filter-candle, (*b*) 250 c.c. were withdrawn and treated by alum process. As in No. 6, numerical comparison was instituted between the two methods by counting number of colonies on the two sets of plates. It was found, (*a*) by filtration, 80 c.c. of water averaged 9 to 10 colonies, (*b*) by alum process, 80 c.c. of water averaged 28 colonies—ratio of about 1 to 3, closely agreeing with result in No. 6.

Two days later, tank water was again examined by alum method. T.b. not found in 100 c.c.

No. 10. Tank with 90 litres of tap water and 180,000 t.b. A few hours after introducing t.b. a sample of water was withdrawn and treated as usual. 40 c.c. of sample yielded 45 typhoid colonies. 24 hours later a second sample of water was withdrawn and treated precisely as before. 400 c.c. now yielded 15 typhoid colonies.

Two days later 40 c.c. failed to yield one t. colony. Six days after introduction of bacilli none were recovered from as much as 400 c.c. of water.

Remark. The rapid dying out of t. b. was probably due to two causes: (i) the presence of numerous other organisms, (ii) chemical or electrical action of the zinc-iron coating of the tank[1]. Twice, after the water had been standing in the tank for two or three weeks, a greyish-white film was observed all over the surface. This film was found on examination to consist of zinc hydrate and carbonate.

No. 11. Tank with 100 litres of tap water to which were added 61,000 coli and 35,000 typhoid. 160 c.c. were withdrawn and examined by usual method. In this instance lactose litmus agar had to be used for plating. This medium proved unsatisfactory, much inferior, indeed, to that of D.-C. Most of the plates were spoilt by the rapid growth of "spreaders." One plate, however, on which a very small quantity of precipitate had been spread, yielded four well isolated colonies, two being coli, and two typhoid.

Remark. Despite some unfavourable circumstances t. b. were recovered from water containing twice as many colon bacilli.

Throughout these experiments there was as a rule no difficulty in identifying typhoid colonies on the D.-C. plates. From time to time, however, a colony was selected at random and tested by the agglutination reaction and on various culture media. In any doubtful case these tests were systematically used. In No. 11, for example, the two colonies isolated on lactose agar were fully tested, their appearance, although typical, not being considered sufficient evidence of identity under the conditions of the experiment.

The strain of typhoid used throughout was isolated post-mortem from the spleen a few months before the experiments commenced. The bacillus was actively motile and agglutinated readily with typhoid serum.

The tap water came from an exposed cistern on the roof of the laboratory and usually contained many bacteria per c.c. These gave no trouble on the D.-C. plates, at 42° C. A few sporing forms, streptococci, and occasionally *B. coli*, were most frequently met with.

After the first experiment a large centrifugal machine was used,

[1] Since the above was written P. W. Bassett-Smith (*Journ. Prevent. Med.* July, 1905) has recorded some experiments on the germicidal action of various metals. He finds that "zinc or iron coated with zinc...after 24 to 48 hours appears to free the water from typhoid organisms," and he considers therefore that a galvanized iron tank is most valuable for storage purposes.

containing 12 tubes, and capable of dealing with 500 c.c. of water at a time.

Conclusions.

Recent methods for isolating the typhoid bacillus have been duly considered, successful results in actual practice have been recorded, and a new precipitation method described. An important question remains to be answered. Out of all these methods and processes which should be chosen to-day for the examination of a water suspected of typhoid pollution? In the routine examination of water for the colon bacillus where a considerable volume has to be searched it is now recognised that the conversion of the water itself into a nutrient medium is the best method to adopt, and there can be no doubt that this is also the best method for the typhoid organism. As already shown, Hoffmann and Ficker have devised a process, dependent on the use of caffeine, by which the typhoid bacillus can be "enriched" at the expense of most other organisms; and further, their process has twice given a successful result in practice. The first choice, then, should undoubtedly fall on the process of Hoffmann and Ficker.

But since caffeine varies in its action and does not favour all races of typhoid bacilli alike, it seems advisable to supplement this process by some further proceeding, to use a popular phrase, "to have two strings to one's bow." Of other methods, precipitation is probably the best, and is certainly the most practicable. I have shown that the alum process is capable of giving good results, and in its chemical details it is rather simpler than Schüder's or Ficker's process.

To sum up. In the examination of a suspected water for the presence of the typhoid bacillus there should be employed side by side the method of Hoffmann and Ficker, and some method of chemical precipitation. This combination would seem to offer the best chance of successfully isolating the organism, thus demonstrating the water to be the actual carrier of infection.

Tabular Summary.

- Exam. of water for T. B.
  - Isolation
    - (1) Filtration.
    - (2) Chemical precip.
      - Schüder's process.
      - Ficker's „
      - Alum „
    - (3) Serum agglutination.
    - (4) Enrichment—Hoffmann and Ficker's process.
    - (5) Cambier's process.
    - (6) Solid media
      - Gelatin (Elsner's, etc.).
      - Bile-salt agar.
      - Glucose and lactose agars.
      - Drigalski-Conradi medium.
      - Endo's medium.
  - Identification
    - Morphological and cultural characters, etc.
    - Specific reactions
      - Agglutination.
      - Pfeiffer's, etc.

A METHOD OF TESTING ANTIBACTERIAL SERA, WITH SOME OBSERVATIONS ON THE IMMUNISING BODIES IN THEM.

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The treatment which Dr A. E. Wright has advocated of certain chronic infections, by the injection of sterilised emulsions (vaccines) of the micro-organism causing the disease, is now well known, but a brief statement of its principles may make the following observations more clear.

Wright has found that the injection of such vaccine increases the phagocytic power of the patient's leucocytes, and that this power of phagocytosis is due to the increase of a substance in the patient's serum which is destroyed by heating to a temperature of 60° C., for ten minutes. This substance is supposed to prepare the micro-organism for phagocytosis, and in its absence the phenomenon of phagocytosis cannot take place. This substance Wright calls "opsonin," and the power of the serum to prepare the micro-organism for phagocytosis is termed the "opsonic power" of the serum. This opsonic power is estimated in the following manner. Equal quantities of the patient's serum, of an emulsion in normal saline of the micro-organism, and of washed leucocytes are mixed together in a capillary pipette, and incubated for a definite time at 37° C. A stained preparation is then made of the incubated mixture, and the average number of micro-organisms in the polynuclear leucocytes is estimated. The result of this estimation is compared with the result obtained from a similar preparation made from one's own serum, which is taken as a standard.

While estimating the opsonic power of the blood of some coccus-infected patients whom I have been treating by Wright's method, it occurred to me that a similar technique would furnish a simple and

efficient test for the quality and power of antibacterial sera in general. Accordingly I obtained some "polyvalent" anti-streptococcus serum and proceeded to test it, adopting Wright's technique.

In all the experiments I used my own washed leucocytes. The streptococci were obtained from a scalp wound in which the suppuration showed a marked tendency to spread. The serum was dated Feb. 1st, 1905, and was first tested on Feb. 6th, 1905.

Experiment I.

Tube I contained a mixture of anti-streptococcus serum, emulsion of cocci and leucocytes.

Tube II contained a mixture of my own serum, emulsion of cocci, and leucocytes.

The tubes were incubated for 30 minutes at 37° C., and the average number of cocci in the leucocytes estimated. The following were the results.

Tube I.	Average number of cocci in 30 polynuclear leucocytes	= 11
Tube II.	" " " "	= 12·3

This shows that the opsonic power of the anti-streptococcic serum of this particular strain of streptococcus was less than that of my own serum.

That this power was very rapidly lost after opening the phial is shown by the fact that on repeating the experiment next day the tube corresponding to tube I gave an average of 4 cocci per polynuclear leucocyte. A fresh phial of the same serum obtained on Feb. 24th gave an average of 0 cocci per polynuclear leucocyte.

These experiments demonstrate the progressive loss of opsonin in serum which is kept for any length of time, and if the opsonic hypothesis of immunity from bacterial infections is correct, it is clear that the serum, in order to be of benefit to the patient, would have to be quite recently drawn off. This would mean that the treatment of streptococcal and other bacterial infections by means of antibacterial sera is outside the range of practical therapeutics, since to obtain the maximum effect the serum would have to be almost directly transferred from the immunised animal to the patient.

Considering this, it seemed possible that the opsonic hypothesis might be brought into line with the hypothesis of haemolysis and bacteriolysis, and that the opsonin, which is heat labile (disappearing on heating to 55° C.) and also time labile, might have similar characteristics to complement or alexin. If this were so there might be in the inactive serum a body, heat stable and time stable, corresponding to immune body, copula, or substance sensibilisatrice, and the inactive serum could be reactivated just as an inactive haemolytic serum can be reactivated.

To investigate this point the following experiments were undertaken.

Experiment II.

In this experiment the first sample of anti-streptococcic serum was again used. Equal parts of my own freshly drawn serum and anti-streptococcic serum were mixed, and used as the serum element in tube I. The serum element in tube II was made by mixing equal parts of my own fresh serum and normal saline solution. In each case cocci and leucocytes were added as usual, and the mixtures incubated for a quarter of an hour at a temperature of 37° C.

(*a*) Tube I. Average number of cocci in 30 p.n. leucocytes = 8·7
Tube II. " " " " = 6·6

The next day the experiment was repeated with an exactly similar result. On Feb. 24th, with the new phial of anti-streptococcic serum, the result was as follows:

(*b*) Tube I. Average number of cocci in 30 p.n. leucocytes = 16·2
Tube II. " " " " = 11·3

The larger number of cocci taken up by the polynuclear leucocytes in this experiment was due to the fact that the emulsion used contained more cocci per unit volume than in the former experiment.

What then are the conclusions to be drawn from these experiments? If nothing corresponding to the immune body were present I expected to find that the results obtained from the two tubes would be similar, whereas if such a substance were present tube I would give a larger average per polynuclear leucocyte than tube II. This actually proved to be the case, and the fact would tend to show that there is present a substance corresponding to the immune body, or cupola of Ehrlich's hypothesis, or the substance sensibilisatrice of Bordet.

These experiments furthermore do not support either of the two rival hypotheses of Wright and Neufeld. According to Wright the substance in the serum which prepares the micro-organism for phagocytosis is opsonin, an unstable body, which in his view directly attacks the micro-organism, and neutralizes whatever may be in it which prevents it from serving as food for the leucocyte. Neufeld's view is directly opposed to that of Wright. He worked with a highly immune serum, obtained from rabbits, by injecting a very virulent strain of streptococcus. If an emulsion of these cocci, either in serum previously inactivated by heating, or in salt solution, were mixed with some of this immune serum which was also inactivated, and white corpuscles added, the whole being then incubated, Neufeld found that the polynuclear leucocytes took up enormous numbers of these cocci. It will be seen that anything of the nature of opsonin or complement was carefully excluded by heating the immune serum, by using inactivated serum or salt solution for making the emulsion of the cocci, and by washing the corpuscles.

The conditions of my experiments with the anti-streptococcic serum were practically identical with those of Neufeld's experiments. The serum was inactive, the emulsion of cocci was made with normal saline solution, and the corpuscles were washed. His ingredients were mixed in a hanging drop, mine in a capillary tube, but in no case in my experiments did any phagocytosis take place unless there was some heat labile substance (opsonin, complement) present. I cannot see any explanation of this divergence of results, unless the mode of action of a highly immune serum differs from that of a less highly immune serum, an unlikely hypothesis.

Neufeld contends that Wright's results ought not to be compared with his, since Wright was working with weak normal serum, while he was working with highly immune serum. The following experiments show, however, that the immunising substances in my own serum and in the serum of a patient in the early stages of immunisation, are similar to those in the immune sera obtained from animals.

Experiment III.

The test organisms were staphylococci. In tube I the serum element consisted of equal parts of my own freshly drawn serum and of my own serum heated for a quarter of an hour to 60° C. The serum element in tube II consisted of my own fresh serum and of the serum of a patient which had been heated for a quarter of an hour to 60° C. I expected to find that if no substance such as an immune body were present the results obtained from the two tubes would be similar, while if such a substance were present the results obtained from the two tubes would differ considerably.

Tube I.	Average number of cocci in 20 Polynuclear leucocytes	= 12·9
Tube II.	,, ,, ,, ,,	= 8·7

Experiment IV.

Tubes I and II made as in the last experiment.

Tube I.	Average number of cocci in 20 Polynuclear leucocytes	8·3
Tube II.	,, ,, ,, ,,	4.8

Experiment V.

The conditions being again similar.

Tube I.	Average number of cocci in 20 Polynuclear leucocytes	4·0
Tube II.	,, ,, ,, ,,	4·4

The patient whose serum was used in this experiment was approaching the end of her treatment, whereas in experiments III and IV the patients were in the early stages of the treatment.

The above experiments show that there is a substance in my own and slightly immune sera which is heat stable, and which has a decided influence in the opsonic power of the serum, and consequently, is presumably of the same nature as the substance in the highly immune sera obtained from animals, in fact is the immune body.

To sum up the results. According to Wright the substance in the serum which prepares the micro-organism for phagocytosis is heat labile; according to Neufeld it is heat stable; while the above experiments seem to show that an interaction of a heat labile with a heat stable substance is necessary for the process to take place.

There remains the practical question as to whether an inactive immune serum is of any use in the treatment of a patient. Experiment II *a* and *b* show that it is, provided that the necessary complement is present in the patient's plasma. It would seem that the effect of this heat stable substance is to concentrate so to speak the inimical complement on the invading micro-organism. In some severe infections, where complement may be almost or completely absent, the injection of inactive or feebly active serum would be useless. To meet this difficulty Wassermann has suggested the injection at the same time of some normal serum, in order to provide complement. There seems to be no reason why this should not be done, since the difficulties would be no greater than in the method of transfusion for the loss of blood.

The next serum tested was some antistaphylococcus "aureus" serum which was sent to me for trial.

Experiment VI.

In tube I was a mixture in equal quantities of the antistaphylococcus serum, emulsion of cocci and corpuscles.

In tube II the serum element was my own fresh serum. Both tubes were incubated for twenty minutes at 37° C.

Tube I.	Average number of cocci in 20 Polynuclear leucocytes	= 0·0
Tube II.	,, ,, ,, ,,	= 9·5

Repetition gave an exactly similar result, and later I obtained another sample of the serum and repeated the experiment with the following result.

Tube I.	Average number of cocci in 30 Polynuclear leucocytes	= 0·0
Tube II.	,, ,, ,, ,,	= 8·4

Experiment VII.

A similar method was adopted with this serum as with the antistreptococcic serum, the serum being reactivated with my own freshly drawn serum. In tube I the serum element consisted of my own freshly drawn serum and the antistaphylococcic serum in equal parts. In tube II the serum element was my own fresh serum and salt solution in equal parts.

Tube I. Average number of cocci in 20 Polynuclear leucocytes = 4·2
Tube II. „ „ „ „ = 7·2

This experiment gives a result the exact reverse of the similar experiment with the antistreptococcic serum (Experiment II (*a*) and (*b*),) showing the presence of some substance having a distinctly inhibitory influence on my serum. That this substance was heat resistant is shown by the following experiment.

Experiment VIII.

Tube I was made up as in Experiment VII except that the antistaphylococcic serum was previously heated for a quarter of an hour to 60° C. Tube II was as before.

Tube I. Average number of cocci in 30 Polynuclear leucocytes = 4·3
Tube II. „ „ „ „ = 7·0

I then tried to find out on which of the elements in my serum this substance was acting. The following experiment shows that the substance present was antagonistic to the complement.

Experiment IX.

The cocci used in both tubes were first incubated with my inactavited serum for half-an-hour at 37° C. To tube I was added a mixture of my own fresh serum and antistaphylococcic serum in equal parts, they having been previously incubated together for a quarter of an hour at 37° C. In tube II the mixture consisted of my own fresh serum and salt solution in equal parts and similarly incubated together. The final mixture in each tube was completed by the addition of corpuscles.

Tube I. Average number of cocci in 30 Polynuclear leucocytes = 3·8
Tube II. „ „ „ „ = 6·3

Repetition of the experiment gave a similar result.

The question remains how this anti-complement is to be accounted for. I am informed by the manufacturers that the serum used was that of a horse which had been immunised by increasing doses of sterilised broth cultures of the staphylococcus, given at intervals of four days, and that the serum was drawn off on the eleventh day after the last injection.

It has been suggested to me that the phenomenon was due to the presence of some preservative added to the serum by the manufacturers. I find on enquiry that a small quantity of trikresol is added to all the sera they supply, so that if the effect was due to the presence of this substance, it ought to have been present in the case of the anti-streptococcic serum. Possibly the explanation may be that the horse had attained a condition of hypersusceptibility.

Conclusions.

1. That there is in inactive immune serum a substance corresponding to immune body.

2. That a like substance is present in my own serum and in the serum of patients in the early stages of immunisation.

3. That the micro-organism is prepared for phagocytosis by the interaction of two substances, one heat labile (complement), the other heat stable (immune body).

4. That an inactive immune serum is of use in treatment provided that complement is present in the patient's plasma.

Whether the above conclusions prove to be right or wrong, I trust the original object of the experiments has been attained, and a simple method of testing antibacterial sera demonstrated.

I am greatly indebted to Professor McWeeney for his kind assistance and keen criticism, both of my experiments and their results.

ON THE PRESENCE OF A SARCOSPORIDIUM IN THE THIGH MUSCLES OF MACACUS RHESUS.

By W. E. de KORTÉ, M.B. Lond.

(*From the Bacteriological Laboratory, King's College.*)

As far as can be ascertained this sporozoon has not been described before as occurring in a monkey, though several cases have been recorded in man[1] and in a number of other mammals.

Unfortunately the monkey was incinerated prior to the discovery of the parasite in the reserved piece of muscle in which it was found, nothing further can therefore be said about the distribution of the parasite in this animal. The features of the sarcosporidium found in the rhesus conform in the main with the known characteristics of this order of sporozoa, whose habitat, with rare exceptions, is the striped muscles of warm-blooded vertebrates. The microphotographs give a fair idea of the appearance of the trophozoite in the intramuscular stage of the parasite.

Photomicrograph No. 1, Plate XII, shows a transverse section (× 500 diameters) of an affected muscle fibre. It shows a capsule consisting of two coats, an outer dense clear sheath, traversed by fine striae, possibly fine canalicules through which nourishment passes from the surrounding lymph spaces to the parasite. These striae are arranged vertically to the surface of the parasite. The inner coat consists of a thin homogeneous membrane, which is not continued internally, however, into the protoplasmic body of the trophozoite. As will be seen from the illustration, the endoplasm consists of minute irregularly circular naked spores packed very closely together. There are no pansporoblasts or alveolar

[1] Smith, T. (1904). *Journal of Exp. Medicine*, VI, pp. 1—21, pls. I—IV. Vuillémin, P. (1902). *C. R. Ac. Sci.* Paris, CXXXIV. p. 1152.

meshwork separating these. The spores appear to be *ab initio* of one kind only, and of the nature of naked gymnospores. If a spore is critically examined, beyond a central clear space, which may be a nucleus, no cell membrane or other structure can be made out. Judging from such appearances as are present, it seems likely that the spores multiply by simple division, and that the clear space seen in some of the spores is the commencement of such division.

Photomicrograph No. 2 represents a longitudinal section (× 100 diameters), it shows the great distension of the muscle fibre by the parasite. The poles of the trophozoite show no differentiation from the body, so that these regions cannot be looked upon as centres of proliferation. It will be further noticed that the affected fibres are not surrounded by any inflammatory small cells, which may in some measure serve to explain the absence of symptoms in the sarcosporidiasis of some animals.

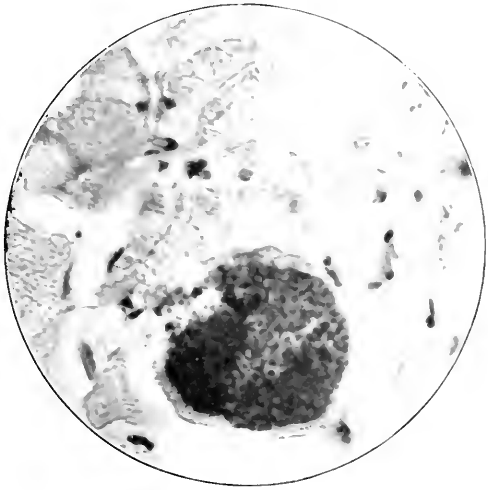


Fig. 1.

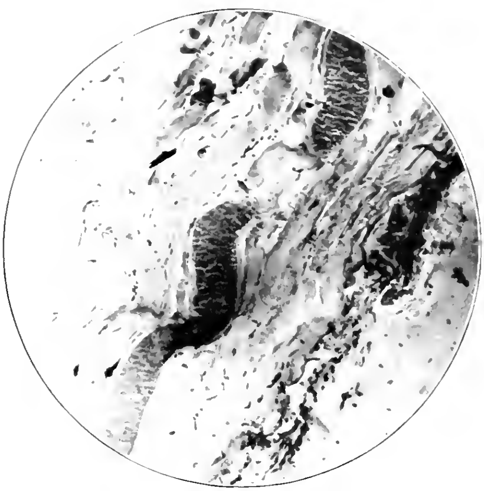


Fig. 2.

A NEW FORM OF PARASITE FOUND IN THE RED BLOOD CORPUSCLES OF MOLES.

By G. S. GRAHAM-SMITH, M.A., M.D.,

John Lucas Walker Student, Cambridge.

(*From the Pathological Laboratory, Cambridge.*)

At the present time when the parasitic protozoa found in the blood are receiving great attention, it may be of interest to describe a form which appears not to have been noticed hitherto.

In examining the blood of various animals for the presence of *Piroplasma* the organisms about to be described were discovered in the blood corpuscles of a mole. In this specimen a considerable number of the red blood corpuscles were found to be infected, and it was therefore thought that by investigating a number of moles other infected specimens might be found.

In all the blood of 102 moles, caught in the neighbourhood of Cottenham, near Cambridge, was investigated. The moles were brought to the laboratory alive, and smear preparations were made from their hearts' blood and from the various organs. The organs were also preserved for histological purposes

Parasites in greater or lesser numbers were found in the red blood corpuscles of ten (10 %) of these moles.

Morphology of the parasites.

The morphological characters of the parasites are best seen in specimens stained by Giemsa's method. They also stain well by Leishman's method.

The parasites appear as longer or shorter rods of irregular contour lying within the red blood corpuscles. The rods are never straight, and many have a marked curve in them, often near one of the extremities.

One or both ends of the longer forms are enlarged, giving the organisms a wedge- or club-shaped appearance. Some of the medium sized forms are definitely dumb-bell shaped, the very small ones almost round.

By Giemsa's stain the protoplasm of the organisms stains a light blue colour, with darker areas at the enlarged ends. In most of the longer forms some portions of the protoplasms stain more darkly than others, giving the organisms a banded appearance. Comparatively large masses of chromatin are frequently found usually situated near one of the dilated ends of the longer or dumb-bell shaped forms. The larger chromatin masses are round or oval in shape. Not infrequently small dots of chromatin can be seen near the ends of some of the organisms. Some of the longer forms, and the majority of the smaller, show no indications of the presence of chromatin.

The longest specimens vary from $\frac{1}{4}$ to $\frac{1}{3}$ of the length of the diameter of the red blood corpuscles, and the shortest are about $\frac{1}{8}$ of the length of the long forms. The length varies between $\cdot 1\mu$ and 1μ.

Parasites are occasionally found free in the plasma. In such cases they are usually in groups as if recently liberated by the rupture of a corpuscle. Isolated free forms were rarely seen.

Degree of infection in the 10 *infected moles.*

The number of infected red blood corpuscles in smears from the heart's blood varied greatly in the 10 infected moles. In the following table they have been numbered according to the degree of infection.

Table showing the degree of infection.

Mole	I.	About 1 % of all red corpuscles infected.	
,,	II.	,, ·3 % ,, ,, ,,	
,,	III.	10 infected corpuscles found containing	8, 9, 22, 28, 30, 30, 32, 32, 33 and 42 parasites.
,,	IV.	4 ,, ,, ,, ,,	6, 7, 20, 32 parasites.
,,	V.	2 ,, ,, ,, ,,	1 and 7 parasites.
,,	VI.	2 ,, ,, ,, ,,	18 and large numbers of parasites.
,,	VII.	2 ,, ,, ,, ,,	18 and 22 parasites.
,,	VIII.	1 ,, ,, ,, ,,	40 parasites.
,,	IX.	1 ,, ,, ,, ,,	Very numerous parasites.
,,	X.	1 ,, ,, ,, ,,	,, ,, ,,

It will be seen from this table that in the blood of moles III to X, in which very few infected corpuscles were found, the number of parasites within such corpuscles was usually very high.

In smears taken from the heart of mole I the number of parasites contained in 1321 infected red corpuscles were counted, and the following table gives the total number of corpuscles containing various numbers of parasites, and their relative proportion to each other.

Table showing condition of infected corpuscles in films from the heart blood of mole I.

No. of parasites in infected corpuscle	No. of infected corpuscles counted	Percentage of each variety	No. of parasites in infected corpuscle	No. of infected corpuscles counted	Percentage of each variety
1	12	·98	19	8	·65
2	7	·52	20	15	1·12
3	9	·68	21	3	·22
4	14	1·06	22	5	·38
5	8	·65	23	1	·07
6	61	4·60	24	1	·07
7	64	4·83	25	2	·15
8	169	12·79	30	2	·15
9	132	9·99	38	1	·07
10	181	13·70	42	1	·07
11	122	9·23	45	2	·15
12	184	13·85	46	1	·07
13	94	7·11	50	2	·15
14	96	7·26	53	2	·15
15	36	2·72	56	1	·07
16	39	2·95	58	2	·15
17	19	1·43	79	1	·07
18	24	1·81		1321	

It will be seen that very few corpuscles were encountered containing between 1 and 5 parasites. Of all the infected corpuscles 94 % contained between 6 and 20 parasites. More than 20 parasites were found in about 2 % of the infected corpuscles. As far as it was possible to count them, 79 appeared to be the highest number of parasites found in one corpuscle.

Corpuscles containing very large numbers of parasites were slightly enlarged, but those containing moderate numbers, up to 20, were not enlarged.

Smears from the organs.

No infected corpuscles were found in smears made from the liver lung, spleen, kidney, or marrow of moles III, IV, V, VI, VIII, and IX. A few infected cells were found in the lung and spleen smears from moles I, II, VII, and X, in liver smears from moles II and III, and kidney

smears from moles I and II. Very numerous infected corpuscles were however encountered in smears from the liver of mole I.

In all cases the number of the parasites within the infected corpuscles was large, and corpuscles containing small numbers of parasites were never met with except in liver smears from mole I. Smears made from the marrow obtained from the shaft of the femur very seldom showed infected corpuscles.

For the sake of comparison with the previous table the number of parasites within 206 infected corpuscles were counted in liver smears from mole I.

Table showing condition of infected corpuscles found in smears from the liver of mole I.

No. of parasites in infected corpuscles	No. of infected corpuscles counted	Percentage of each variety	No. of parasites in infected corpuscles	No. of infected corpuscles counted	Percentage of each variety
2	4	1·8	12	27	13·1
3	1	·4	13	16	7·7
4	8	3·9	14	9	4·3
5	2	·9	15	7	3·4
6	10	4·8	16	7	3·4
7	11	5·3	17	2	·9
8	23	11·1	18	2	·9
9	28	13·6	19	1	·4
10	33	16·0	20	1	·4
11	13	6·3	48	1	·4

Autopsies.

No marked macroscopical lesions were found by which the organs of infected moles could be distinguished. Enlargement of the spleen was present to a marked degree in 12 moles, and of these one was found to be infected. A nodular cirrhotic appearance of the liver was noticed in two moles and cysts in the liver in two others. None of these were infected.

Histology of the organs.

The organs of all the infected moles were examined and compared with the organs of normal moles. No changes were discovered in the spleen, kidney, or heart muscle. In the lungs large collections of polymorphonuclear leucocytes were in some cases seen around the bronchi and larger vessels. In some of the infected moles there was a bronchopneumonic condition. Similar changes were seen, however, in the lungs of moles in whose blood no infected corpuscles were found.

The livers of all ten infected moles and of eight apparently healthy moles were examined. All but two of these were infected with *Coccidia* (90 %) in the bile-ducts. No other changes were found in the livers of moles III to X.

In the case of moles I and II, however, which showed the greatest degree of infection in the blood, the livers exhibited marked changes, probably therefore resulting from infection with this organism.

Liver of mole I.

Throughout considerable areas the substance of the liver appeared to be normal, and when stained by haematoxylin and van Gieson's stain the cells were well defined with yellowish protoplasm, and compact, round, blue, darkly staining nuclei. The appearance of the normal liver is shown in Plate XIV, Fig. 1.

Several degenerated, badly staining areas can, however, be seen in sections cut from various parts of the liver. In passing from the healthy liver substance towards the degenerated portion the following changes are seen. First the protoplasm of the cells begins to stain poorly, and the cell outlines become indistinct. A little further the cell outlines are lost, and many of the nuclei are badly stained. Here and there, however, in these areas are groups of cells or isolated cells, which still stain well and show apparently normal nuclei. Plate XIV, Fig. 2, represents the appearances seen in such areas as have just been described. Still further the remains of the nuclei can only be made out with difficulty, and small, rounded, clear spaces can frequently be seen in the granular remains of the cell substances. A badly staining granular mass, without a trace of cells or nuclei, is usually to be found in the centre of the affected area.

Into some of these degenerated areas haemorrhages had taken place.

In the liver of mole II the condition was much the same as in that of mole I, but was not so advanced. Completely degenerated areas in which the cells had totally disappeared were not found. In one lobe of the liver extensive haemorrhages, both old and recent, had taken place into some of the degenerated areas.

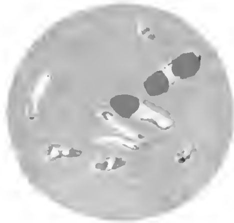
In suitably stained sections no bacteria were found, and sections treated by Leishman's and Giemsa's methods did not reveal the presence of parasites.

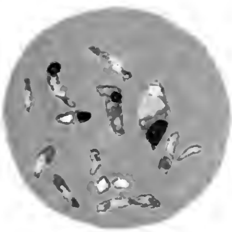
External animal parasites on moles.

The external animal parasites were recorded in all the moles which were investigated. Fleas and lice were found on all of them, mites on a considerable number, but ticks were never seen.

The parasites described do not appear to have any relation to *Piroplasma*. They differ markedly in morphology and in the numbers which are found within infected corpuscles. In the peripheral circulation in the course of the disease due to *Piroplasma canis* of all infected corpuscles those containing a single parasite are the most commonly found, but in this disease corpuscles containing less than six parasites are rare. In the former disease the parasites are usually found in even numbers in the greatly infected corpuscles, whereas in this case corpuscles containing odd numbers of parasites are nearly as common as those containing even numbers. In the former disease great multiplication takes place in the marrow, but very few parasites were encountered in smears made from the marrow of the infected moles.

Owing to the difficulty of keeping the animals alive for any time no observations were made on the transmission of infection, or the effects or duration of the disease.





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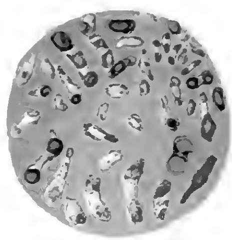

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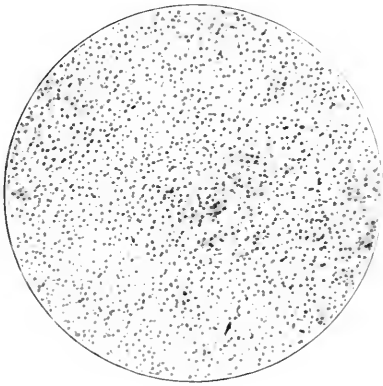

Fig. 1

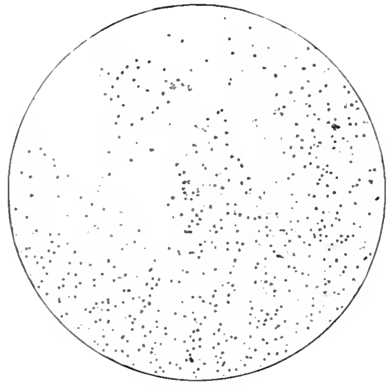

Fig. 2.

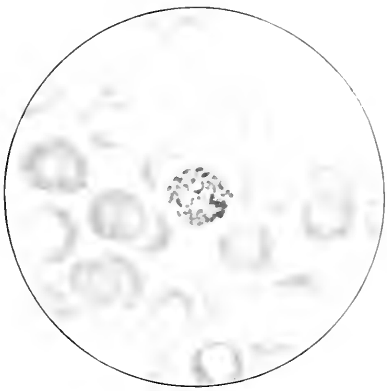

Fig. 3.

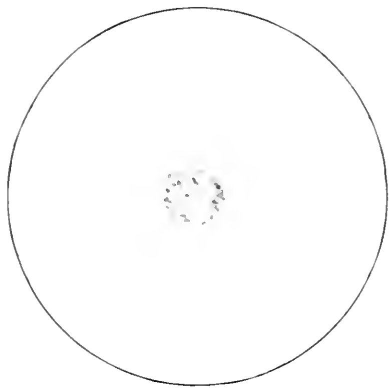

Fig. 4.

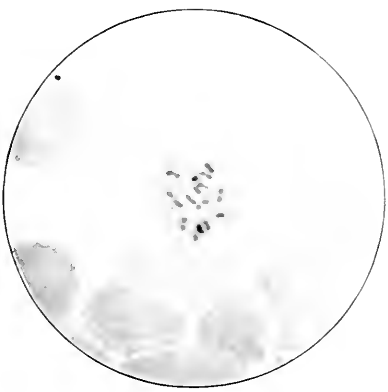

Fig. 5.

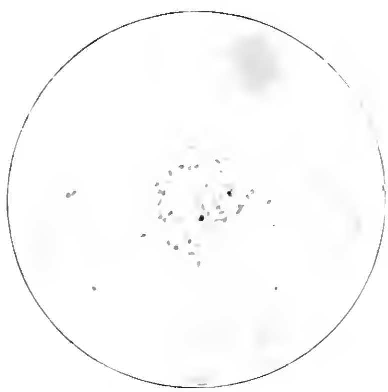

Fig. 6.

EXPLANATION OF PLATES XIII AND XIV.

PLATE XIII. The figures represent the parasites as seen within the corpuscles of mole II in specimens stained by Giemsa's method. The differentiation between the chromatin masses and the rest of the protoplasm is well shown.

Fig. 1. Red blood corpuscle containing 2 parasites.
Fig. 2. ,, ,, ,, ,, 9 ,,
Fig. 3. ,, ,, ,, ,, 14 ,,
Fig. 4. ,, ,, ,, ,, 19 ,,
Fig. 5. ,, ,, ,, ,, 29 ,,
Fig. 6. ,, ,, ,, ,, 39 ,,

PLATE XIV. Fig. 1. Photograph (× 100) of normal liver substance of mole I.

Fig. 2. Photograph (× 100) of a portion of the liver of mole I near a degenerated patch. On one side of the specimen a considerable number of nuclei are still distinctly stained and the cells are distinguishable. Passing towards the degenerated area the cells become less distinct and only small numbers of the nuclei distinctly stained. The most advanced stage of degeneration is not here represented.

Fig. 3. Photograph (× about 1500) of a greatly infected red blood corpuscle partly overlying a normal corpuscle. About 50 parasites are present in this corpuscle. (Stained by Giemsa's method.)

Fig. 4. Photograph (× about 1500) of another greatly infected corpuscle. (Stained by Giemsa's method.)

Fig. 5. Photograph (× 2000) of a red blood corpuscle containing 15 parasites. (Stained by Leishman's method.)

Fig. 6. Photograph (× 2000) showing 59 free parasites in a group. (Stained by Leishman's method.)

I am indebted to Mr Walter Mitchell for these excellent microphotographs.

THE MALARIAL FEVERS OF JERUSALEM AND THEIR PREVENTION.

BY JOHN CROPPER, M.A., M.D. (Cantab.),
Ramallah, Jerusalem.

IN a previous paper[1] I referred to observations by Dr E. W. G. Masterman, on the prevalence of malaria in Jerusalem. Since that time Dr Masterman has searched various places for Anopheles in and near the city, and has found numerous larvae in the Birket Mamilla (or "Upper Pool of Gihon") in winter, the water of which is conducted by covered conduits to Turkish baths in the city. The pool is dry in summer.

The Pool of Hezekiah, near the Jaffa gate, contained no Anopheles larvae in the spring of 1903, but in various rain-water cisterns inside the walls larvae were almost invariably found and imagines in the London Jews' Society's Hospital. Last year Dr Wheeler found Anopheles in six Jewish houses in the city, and I found them in five, but only in dark rooms, in the hospital above mentioned, and also in many cisterns in and outside the city.

Having carried on part of Dr Masterman's work during his absence from September, 1904, to January, 1905, inclusive, at the London Jews' Society's Dispensary inside the city, and practising in the neighbouring village of Ramallah on the other days of the week, I have had exceptional opportunities of observing the malarial fevers, not only of Jerusalem, but also of the surrounding country. During the period stated I examined the blood of about 500 cases of fever microscopically.

Jerusalem, from its natural position, 2500 feet above the level of the sea and 3300 feet above the Jordan valley, surrounded on three sides N., E., and S. by deep limestone valleys, without streams of water, with

[1] Cropper, J. (1902), *Journ. of Hygiene*, vol. II, pp. 47—57. "The Geographical Distribution of Malarial Fever and Anopheles in Upper Palestine." (See p. 56.)

almost no springs, and absolutely no marshes within a radius of 20 miles, should, if any city, be beyond a suspicion of malaria[1]. The exact contrary of this is the case, as will appear below: few spots, even on the West Coast of Africa boasting a higher endemic index, than some at least of the Jewish colonies of Jerusalem.

A very large number of Jews live in or near the city. No census is possible, but 40,000 is probably a reasonable estimate of their numbers at the present day. Though most of the facts adduced below refer to these people, I have good evidence of the occurrence of malaria amongst the Arabs, Europeans, and others living in the city.

The Jews inside the city are mostly Sephardim or Spanish Jews, at once the poorest and most despised, but far the cleanest in their habits; they inhabit for the most part the Jewish quarter which occupies the S.-E. corner of the city, and extends from the Haram or Temple area to the Syrian and Armenian Convents.

The so-called Jewish "colonies" outside the city, over 40 in number, occupy every possible position from the lowest named colony Silwân (Siloam) to the highest Abu Bussal on the Jaffa road, and the Bokhara colony near by, 2650 feet above sea-level. Some have an eastern aspect, others face due north, and in fact every direction conceivable. Some are in hollows, some on steep hill sides, or in exposed positions. Nearly all have equally dirty surroundings, scavenging being nearly unknown, the Bokhara colony being a notable exception. The "colonies" have for the most part been erected by small limited companies, and the houses are generally one or at the most two storeys high, overcrowding is well-nigh universal.

One factor alone, out of many, which seems to have any real influence on the incidence of malaria, is the presence of cisterns of water. I mention this because the only colony where I found splenic enlargement quite rare amongst the children was (at the time of my visit) without a single cistern, water being brought from a distance. This defect is now being remedied, but I did not know of the fact until after my visit. At the present time the population of Jerusalem depends for its water supply almost entirely on rain water stored in cisterns cut out of solid rock; Anopheles breed almost entirely in these cisterns.

The average rainfall of the past 22 years was $28\frac{1}{3}$ inches (see

[1] So scarce is water that at the end of summer from £5 to £10 can be readily obtained for a cistern of water.

Quarterly Statements of the Palestine Exploration Fund). The hot months being May—October, and the wet months November—April.

The following statistics will give some idea of the prevalence of malaria among the Jews, who form quite $\frac{2}{3}$ of the total population. Of 937 new out-patients seen at the Dispensary (of the L. J. S.) in the city from Sept. 13 to the end of the year 1904, no less than 424 were diagnosed as suffering from malaria. Of these cases 228 had enlarged spleens, and in 80 malarial parasites were found microscopically. The spleen was not examined in every case, and on no occasion were more than 11 slides examined, owing to lack of time and other causes. As a rule fresh films were used, but in a considerable number of instances slides were stained by Leishman's method, and the results thus checked.

On Oct. 11 of 60 patients seen in the city 30 were diagnosed as malarial. Owing to the large number who had previously taken quinine the positive findings were more numerous than might have been expected. During the same period 179 cases were examined at Ramallah with positive results.

The percentages of the various forms of malaria found out of a total of 259 positive examinations work out approximately as follows:

Tertian	= 20 %	
Quartan	= 4 %	
Remittent or Tropical	= 68 %	Double Infection = 8 %
Doubtful (Pigmented leucocytes —broken parasites, etc.)	= 8 %	
	100	

Every effort was made not to count any patients' names twice. Several were examined 2 to 3, or even 4 times. The number of the double infections would probably have been increased had more time been available.

Crescents were seen in 13 cases, and exflagellation in all forms of fever, including quartan. This is hardly the place for discussion of the various forms of the "tropical" parasite, but by far the commonest form was not that of pale rings so often described and so easily seen and stained, though these occurred in over 10 %, but the very minute oval, rod, or pear-shaped bodies, occasionally showing active though slow (hardly amoeboid) movement[1].

[1] See J. Cropper (1. v, 1905). "Note on a form of malarial parasite found in and around Jerusalem. *Journ. Tropical Med.* vol. VIII, pp. 132—133. One figure."

This form is very common in children (50 %), and is accompanied by a considerable amount of splenic enlargement, being often very resistant to quinine, though it yields in the end. It is occasionally found in persons evidently suffering from malarial poisoning without actual fever.

Crescents are rare in this infection. This was the only form of malarial parasite found in the case of an Armenian boy who died in the hospital in November from pernicious fever, with well-marked tetanic symptoms. In the past 10 years, as I am informed by Dr Wheeler, there have been three deaths from pernicious attacks. Almost all the cases showing typical pale rings have come from Jerusalem, the Jordan valley, or villages to the east of Ramallah, whose people often visit the valley. Several cases have also occurred amongst men who went to work on the new Haifa Damascus railway near Tiberias.

I think from what I have seen that it will be proved that those who are quite immune to the fever occurring in the hills at once fall victims to the fever of the Jordan valley. Anopheles is present in sufficient numbers in both places.

In three cases of remittent fever of a severe type I observed the parasites sporulating in the peripheral blood (of the ear).

The youngest patient whose blood I have examined was 20 days old; in this case the spleen was considerably enlarged. I have seen several patients aged 3 and 4 months in whose blood the parasite of tertian or remittent fever was found. Evidence of the occurrence of malaria amongst the non-Jewish population has chiefly come to me from those who have come to Ramallah for their health after suffering from malaria in Jerusalem, these cases fully bore out what I found obtaining amongst the Jews, and presented nothing of special interest.

Want of time has alone prevented me from making a much more extensive examination of the Jewish colonies, and the following facts are only given as a fair sample of what may be found.

In October, 1904, I examined a corner of the Damascus Gate colony containing 8 houses. Of 15 children every one had notable splenic enlargement. The older people had every appearance of chronic malaria.

The children's ages ranged from 3 months to 15 years. In one house where Anopheles was quite abundant, the father and mother, and five children aged 3 months to 15 years, all had enlarged spleens, and on this and on subsequent occasions I found malarial parasites (tertian and remittent) in two of the family aged 5 years and 3 months, and a clear history of quartan malaria in the father.

I moreover visited the colonies of Mia Shârim and Oel Mosché. At Mia Shârim of 33 children examined aged 2–14 years, 20 had enlarged spleens. At Oel Mosché I visited several schools of children aged 3–14 years; of 104 children, 36 had enlarged spleens, but the children in these schools came undoubtedly from the most healthy and breezy suburbs of Jerusalem, and moreover those at the time suffering from fever were probably not at school. On one occasion (Oct. 11th) I treated at the Dispensary in the city 8 girls from the same school suffering from fever—in all of these the spleen was notably enlarged, and from what I can judge there is more malarial saturation as a rule inside the walls. It is perhaps worthy of note that in every house where Anopheles was found, there were patients down with fever (with only one exception). A few dissections of mosquitoes were made, but not enough to be of any real value. Judging by previous records the months of the greatest incidence of malaria are September, October, and November—when the proportion of malarial to other cases is $\frac{1}{3}$ to $\frac{1}{2}$. After the rains and cold weather have set in the number gradually diminishes; and in February, March, and April the proportion is $\frac{1}{8}$ to $\frac{1}{5}$ of the whole, after which the number increases until September The rains cease in April, hence malaria coincides with the hot weather and not with the rainy season as in other parts.

Concerning the habits of Anopheles, one thing has been most prominently brought to my notice, and that is that *Anopheles during the daytime will select the coolest, darkest and dampest spot available, especially avoiding well-lighted, whitewashed rooms. Where the houses are the coolest and the darkest hiding-place to be had they will be found in these, but not otherwise; they may always be found by day in the cisterns.*

At first we were greatly puzzled by the rarity of *Anopheles* in the houses by day, *Culex* being always much in evidence, and especially *Stegomyia fasciata*, etc. The reason for the above scarcity was not apparent to me, until, having an occasion to examine a cistern at Ramallah which was being cleaned out, I found (on descending into it by day) numerous Anopheles flying round. The water at the bottom of the well contained the larvae in abundance, but none of *Culex*. Osler's statement therefore that Anopheles is a country insect is hardly true, for in Jerusalem, where the only species is *A. maculipennis*, this is at least equally as common as in the country, and probably more so. The comparative immunity enjoyed by the peasants I attribute to:

(1) The scarcity of cisterns, all water being sometimes carried two or three miles, at any rate in summer. In Ramallah there are 50 cisterns for 5000 people.

(2) Less overcrowding.

(3) Healthier open-air life.

(4) Exposure to breezes at night, which prevents mosquitoes flying.

(5) A relative condition of acquired immunity.

The last statement needs to be qualified, for peasants from the hills on visiting the Jordan valley, as already stated, often acquire a most virulent form of fever, which the Arabs call "plague" (or "wakhm"), which reduces them to a condition of great prostration if untreated: this frequently is bilious remittent in type[1].

At Jericho there is evidently much endemic malaria, and enlarged spleens are common.

Prophylaxis. Could any real pressure be brought to bear on the authorities the prevention of malaria in Jerusalem would be an easier matter than in any part of the globe. There are no marshes to be reclaimed, no streams to be diverted, no puddles to fill up, and few open collections of water, and these easily dealt with. Hitherto efforts to influence the Government have been in vain. The following methods have been suggested by Dr Masterman[2]:

(1) To close the wells.

(2) To use pumps instead of buckets.

(3) The systematic use of quinine throughout the city.

The first measure is of course impossible of achievement where several families use the same well. The best closed well I have seen

[1] Early in July, 1904, five healthy men went down from this village (Ramallah) to work on the Haifa-Damascus Railway in the Jordan valley near Beisan. In 17 days (almost exactly) they returned all ill with fever, bilious vomiting, etc., the illness having lasted about a week. Three of them were well enough to come to the dispensary here for medicine, the other two were seen in bed in their houses. The native doctor here pronounced them all to be cases of enteric fever. The blood of one of them (temperature 102° F.) on examination showed a fair number of crescents. All the cases were therefore diagnosed as bilious remittent fever and all recovered in a few days under quinine, at least 20 grains daily, and, so far as I know, only one case relapsed later. In all probability all of these men had had the form of malaria so prevalent here in infants; amongst the latter crescents are practically unknown, though splenic enlargement is common. This corresponds with what A. Plehn found in Africa as quoted by Manson (*Tropical Diseases*, ed. 1900, p. 66).

[2] In his lecture on the sanitation of Jerusalem, *Pal. Expl. Fund Quarterly Statement*, Jan. 7th, 1905.

when opened revealed Anopheles inside. The second measure is impracticable, for the poorest classes of Jews *cannot*, and the landlords *will* not, go to the expense of pumps, and these would soon be out of order. The third measure has been tried as thoroughly as possible for many years. In the last 10 months at one hospital no less than 600 ounces of quinine have been used, entailing a cost roughly of £50. There are at least four or five other hospitals probably spending as much, and yet from the statistics available I can find no appreciable diminution in the number of cases of malaria. Moreover young children will not take quinine, when once their fever has gone.

The only feasible method of dealing with the matter in practice appears to me to consist in the *sulphur fumigation of the cisterns*. This is both cheap and easily carried out, and is found not at all to affect the water for drinking purposes[1].

Lastly, I may mention the suggestion of the British Consul in Jerusalem, Mr John Dickson, that of putting an eel into the cistern, as is done, he informs me, in Tunis in N. Africa.

In order to draw the attention of the Jewish authorities to the above, a paper in Yiddish and Judaeo-Spanish is being printed, setting forth the method of the conveyance of malaria and its prevention. We have been treating the wells of private residents by sulphur fumigation. Some of the wells of the German colony at Jaffa were petrolized last summer with very gratifying results, namely, the almost total disappearance of mosquitoes. I was able to convince myself of this when I was there in October.

[1] A bucket containing some live charcoal and a few handfuls of sulphur is lowered into the cistern, which is then tightly shut up and left all night.

THE EPIDEMIC OF MALARIAL FEVER IN NATAL, 1905.

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Natal, which has been considered of recent years to be a malaria-free country, has been visited in the past six months by an extensive epidemic of this disease.

Variations in the extent of the prevalence of malarial fever in different years, and the occurrence of epidemics in regions where the disease is known to be endemic, have been frequently observed in different parts of the world. Numerous instances are recorded of the extension of malaria beyond its normal endemic areas and also of its subsequent recession from districts in which it is not usually encountered. There are instances on record of the appearance of malaria in epidemic form in parts where it has almost certainly not existed before, the disease subsequently becoming established as a dominant factor in the pathology of the district. The most notable instance of such an occurrence is found in the history of the Island of Mauritius, regarding which information may be found in Clemow's *Geography of Disease,* Manson's *Tropical Diseases* and other works.

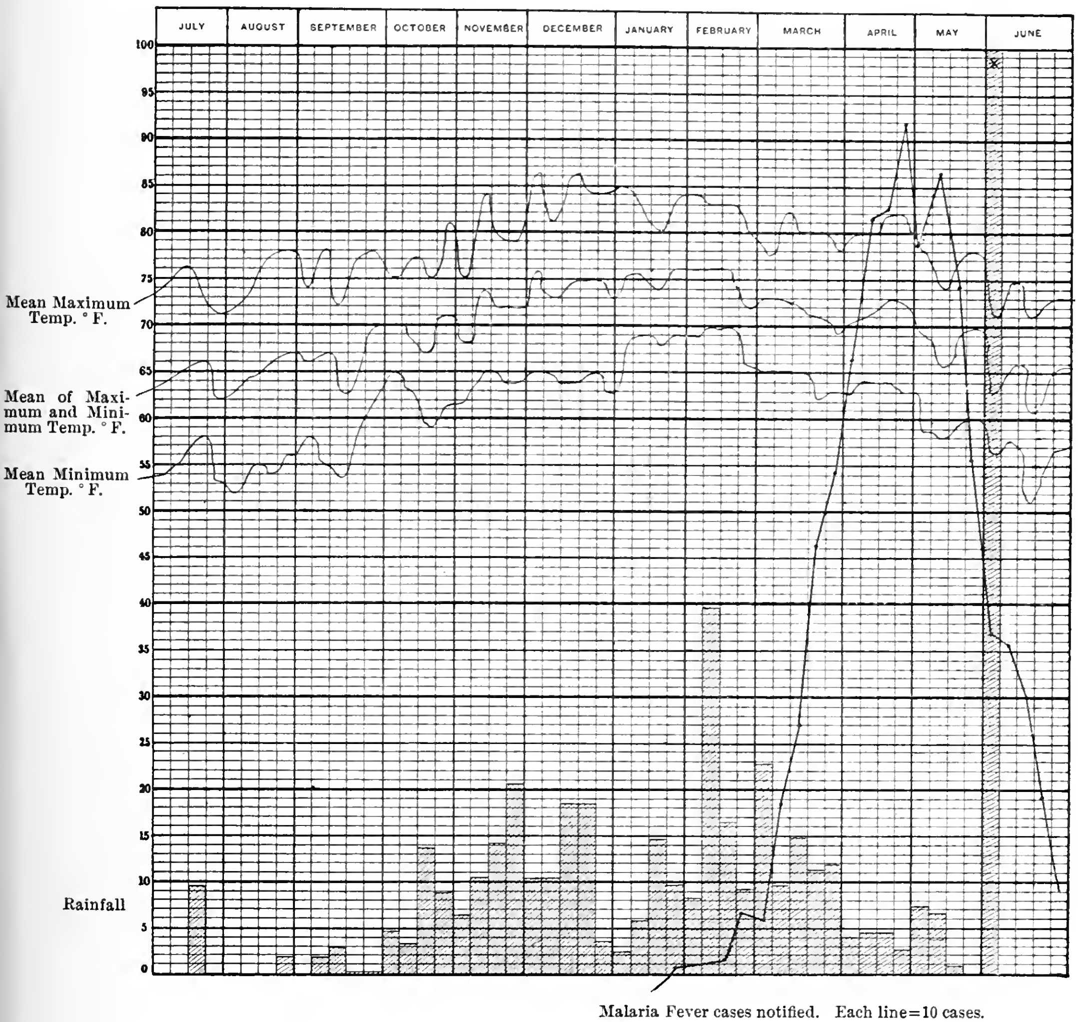
We are not however aware of any reports of important extensions of malarial fever beyond its established endemic areas since the part played by the mosquito in the spread of infection has been established. For the most part such instances in the past have apparently been associated with exceptionally wet seasons, whereas the Natal summer 1904—5 was not remarkable for any particularly lavish rainfall. Of four Districts in which the incidence has been most severe, in three the amount registered has varied during the past summer less than one inch

on the average of the nine preceding years, and in the fourth has been much below it, while the general character as shown by the number of days on which rain has fallen, affords no evidence of higher humidity. A careful comparison of the weekly rainfall in the summer months (November—March) of 1904—5 with that of the preceding three years, so far as records are available, on the higher lands near the coast (which would affect the dimensions of the smaller streams), shows a fall below the average in November and December, a rise above it in January, the rise being about double in February, and in March the point reached somewhat higher than usual. The epidemic reached its fastigium in April (see Chart), which bears the relation to maximum rainfall commonly observed; but in so far as any excess of rainfall might be thought to be a main cause, by increasing facilities for breeding of *Anophelina*, it is noticeable that although it was above the average of three immediately antecedent years, yet the monthly difference as against one or another of those years is hardly so remarkable as to produce such a result, although probably contributing to it; the epidemic had moreover attained considerable dimensions before the rainfall of February (the only marked excess) could have begun to take effect.

The malaria epidemic in Natal has been of notable proportions, and under the circumstances it appears to us desirable to publish this contribution to the epidemiology of the disease. We are unable to advance any sound reason to account for this epidemic, and propose only to set out such slender facts as we have, and to briefly draw attention to their bearings.

The territory of Natal lies between latitude 27° and 31° S., the northern part, still known as Zululand, occupying 27°—29° S. It is divided from Portuguese East Africa on the North by the Pongola River. Inland from the coast line the country rises rapidly to an elevation under the Drakensberg Range of some 5000 feet above the sea, to some degree in plateaux, the first being about a level of 2000 feet. The climate along the coast is sub-tropical, with a mean monthly temperature in the summer months of 70°—80° F., the mean maximum 80°—90° F., with a minimum 15° to 20° lower. The average rainfall in the Coast Districts for the sixteen years 1885—1900 has been 38·44 inches, the highest 71·27, and lowest 27·24, the great bulk of which falls between October and May, January and February being as a rule the wettest months. Thunderstorms are frequent. There is some but not very much marshy ground, mostly about the last few miles of the courses of rivers, which invariably terminate in a tidal lagoon, often sandlocked;

Chart showing weekly notifications of malaria, mean weekly temperatures recorded at Durban, weekly rainfall in district most affected, together with average of weekly rainfall recorded at three stations on the higher ground at the back of the district.



* An extraordinary storm : during 15 hours over 15 inches of rain fell in some parts.

the river beds are much wider than is needed for the average flow, and intermittent streams, as would be expected in a broken country, are general. There is only one town on the coast line of two hundred and fifty miles, namely Durban, with a population of upwards of sixty thousand, composed of Whites, Indians, and Natives of South Africa, of whom the first form nearly half the total. There is a considerable area of marshy ground on one side of Durban, and some parts are low lying and favour the formation of pools. The most important country industry is sugar-planting, and some tea is grown, for the cultivation of which Indians imported under indenture are employed; there are also several thousand Indians composed of coolies who have remained in the country after expiration of their term of indenture, and in lesser number, of traders and artificers.

Malarial fever has always been considered an important disease in Portuguese territory and the northern parts of Zululand. It occurs mostly on the coast, but also extends a considerable distance inland in districts bordering the large rivers. Variations in prevalence from year to year are recorded, as elsewhere; for instance Clemow states that in 1893 malaria was prevalent throughout the Transvaal, but that in 1894 very few cases were recorded. In 1904 nearly all the persons working at a colliery in northern Zululand suffered, in 1905 nearly all escaped. These records however are generally based on the incidence on Europeans, and take little account of the immense Native population. In 1905 malarial fever appears to have been exceptionally severe at Dar es Salaam in German East Africa, as recorded in a local paper. The previous history of Natal in respect of this disease lacks the clearness which is desirable for the purpose of determining the causation of the epidemic of 1905. This is partly due to the want of medical evidence regarding some parts of the country, and partly to a conflict of medical testimony, which is further deficient, in that it is but rarely that the blood has been microscopically examined for purposes of diagnosis. According to popular repute, malarial fever was common in the town of Durban in the sixties and early seventies, indeed we are informed that some old colonists say it was as bad in the "early days" as Delagoa Bay has been considered within recent years, though the conditions there have very much improved of late. As to this we have been unable to obtain professional evidence, but in a volume of personal experiences entitled *Incwadi Yami* Dr J. W. Matthews (then holding a public appointment at Verulam, a coast village less than twenty miles north of Durban) relates that in 1869 malarial fever or bilious remittent

was epidemic in the neighbourhood on the sugar estates. This followed a disastrous flood. In later years, Durban, the country to the south of it, and thirty miles to the north, were considered non-malarious by medical men with one or two exceptions. Where the disease was observed it occurred in persons who had been infected either in north Zululand or in other territories. From those who hold that these parts are not entirely free from malaria we learn that occasional cases, of what appeared to be malarial fever, have occurred during the last ten or twelve years in persons who could not have been infected elsewhere. These cases came to them for treatment. The diagnosis however was not confirmed by blood-examination. The Government Medical Officer of the District situated between Tongaat River (latitude 29° 33′ S.) and Tugela River (latitude 29° 14′ S.), who has held his appointment for over thirty years, assures us that malarial fever is and always has been indigenous in this region. He however states that since 1880 the cases have not been numerous, and the type has been mild. In respect of Zululand, the coast population consists almost entirely of aborigines, who do not to any extent seek medical advice. The Native Commissioner, whose experience of Natives is long and unique, informs us that north of the Umhlatusi River (latitude 28° 45′ S.) malarial fever prevails among them more or less all the year round: that they suffer to some extent in the summer in wet seasons, as far south as Matikulu River (latitude 29° S.): but that he is not aware of fever occurring further south. Within more recent years, several persons were treated for malaria in the Boer Refugee Camp a few miles from Durban, but we are unable to obtain any evidence which would enable previous infection elsewhere to be definitely excluded. In a midland district, one Government Medical Officer is of opinion that malaria occurs in a mild form among natives living along some of the river valleys. A number of cases of malaria are entered in the various hospital records, and since the disease was made notifiable in 1902 have also been notified. A good proportion of those treated in Durban have been sailors from ships in harbour. It may be taken that the majority of all cases notified have, within more or less recent times, been resident in, or visited territories in, parts of which malaria is known to be endemic. Thus Indian coolies and many Whites have admitted that they suffered from previous attacks elsewhere. It has therefore always been assumed that these persons were infected before arrival in Natal, but it is possible that this assumption may have been carried too far.

It has been suggested that the cases recorded in the sixties may, in

default of microscopical evidence, which was of course not at that time available, have been enteric fever. This possibility cannot be doubted, for in some parts where malaria is endemic, many cases of enteric have been classed with the former disease. Nevertheless any such argument necessarily cuts both ways, and is equally applicable to the numerous cases of miscellaneous febrile illness, which, as will be presently shown, have been entered on hospital records of late years.

It is now generally accepted that the diagnosis of malaria in individual cases where the classical signs of periodicity and ague are absent, cannot be satisfactorily made without an examination of the blood; this is particularly emphasised by Manson in *Tropical Diseases* in respect of pernicious fevers, because of the urgency of treatment; but apart from that it is equally applicable to relatively mild attacks such as may be frequently encountered. The diagnosis by clinical symptoms is certainly not always an easy matter. In the *Indian Medical Gazette*, February 1905, M. Watson of Selangor states that sixty per cent. of a series of cases of quartan malaria under his charge in the gaol, and therefore under fairly favourable conditions for observation, were clinically unrecognisable as such. This absence of any characteristic signs was found also in many very mild cases of benign tertian seen by us at the commencement of the epidemic, so much so, that some planters expressed surprise at the statement of the Medical Officers that malaria was assailing their employees. They stated that slight febrile illness of similar character occurred, to a greater or less extent, every year about January or February. Difficulties in diagnosis are naturally enhanced by the circumstances under which coloured persons are seen, especially when all conversation needs to be conducted through an interpreter, and the patient's own description is often the only basis on which it can rest. In such cases unless temperature records are accurately kept, in default of pronounced ague fits, the periodicity of an attack of short duration may readily be missed. In looking through the Annual Reports for past years of the different small hospitals maintained for the treatment of Indian immigrants, there were three points which particularly arrested our attention:—

(1) the large number of cases in some hospitals entered under headings of different febrile illnesses of no pronounced clinical characteristics, such as influenza, dengue fever, fever, febricula;

(2) the generally small number of entries under malaria, which was a little surprising in that among persons coming from various parts of India, a certain number of relapses might have been looked for;

(3) the difference in classification adopted in different hospitals, and in the same hospital in different years, generally concurring with a change in Medical Officers.

In a period of ten years, according to the record of one hospital, cases of malaria were treated every year, but of the other ailments mentioned none occurred excepting in one year, when the number of malaria cases was very small. In two hospitals malaria was never once entered, but in each hospital in one—but not the same—year 11 °/₀ of the cases were classed as fever or febricula. In one of these hospitals, in another year, just under 17 °/₀ of all cases were entered as febricula and dengue. In respect of fever and febricula, at any rate, it is admissible to say that the cause may have been malaria equally well with any other.

The hospitals above mentioned are managed under the orders of Medical Officers, by qualified "compounders," with coolies apportioned for hospital attendance. Temperature charts etc. are naturally not kept in most of these hospitals with the regularity and accuracy observed in modern institutions. In the Government Hospital in Durban, which is conducted in all respects in accordance with the best present day usage, it is most improbable that any but an occasional atypical case of malarial fever could be overlooked. It is not unreasonable in this instance to suppose, that although the disease occurred occasionally and sporadically on parts of the coast, there might be no malaria in the town itself.

It is not possible to say whether or no any of these cases were really malarial fever, or to pronounce definitely whether the country has been free as supposed, or whether the disease has been present all along though unsuspected. If the latter hypothesis be correct, the number of cases must have been relatively insignificant, the pathognomonic signs generally absent, and the type mild. The absence, or at any rate relative paucity, of cases of malarial fever in Natal for many years past, renders the epidemic of 1905 the more notable. Between the last week in January and the end of June 9106 new cases were notified, and 107 deaths registered as directly or indirectly due to malaria. Of these cases 4177, with 42 deaths, occurred in Durban. The racial distribution was as follows:—

	Cases notified	Deaths registered
White races	3234	21
Indians	5404	86
Natives	468	

In considering the incidence on populations living in the affected

districts, it is desirable to eliminate Natives, otherwise a false impression of relative racial immunity might be created; for two reasons:—

(1) the population can only be estimated by districts or counties, and whereas in the districts involved Europeans and Indians live mostly within a few miles of the coast line, and largely in or near the valleys, resident Natives have their kraals on the higher grounds and mostly on the top of knolls, where they are less exposed to attacks of *Anophelinae*. Native families moreover live apart, each in their own kraal, and do not congregate in villages;

(2) Natives working in towns are not prone to seek medical advice, and, not having their families with them, prefer if possible when ill to go home, and so they escape notification. The deaths among natives are fairly well registered, but the cause of death can rarely be determined.

In Europeans and Indians, the incidence of cases notified was reckoned as follows for the population of the districts in which malaria occurred:—

	Per 1000 Population	
	Cases	Deaths
Europeans	78	0·5
Indians	79	1·26 [1]

Outside the Borough of Durban it was only possible to make blood examinations in a fraction of the cases. It is therefore possible that all cases notified were not really malarial, but considering the prevalence of the disease, and its typical character in a large proportion of the cases when the epidemic became well established, it is probable that the margin of error is not wide.

The first cases were notified in the last week of January in the town of Durban, but it was not until the beginning of March that notifications were received from the country districts. Further enquiry elicited the fact that an exceptional amount of minor febrile illness had occurred among plantation coolies, in January and February. Indeed when the occurrence was first reported to the Department of Public Health in the middle of February, and investigation was immediately instituted and blood films examined, the character of the illness in general was so mild, and the clinical symptoms so indefinite, that without blood examination

[1] The death-rate is probably much higher, because it is only the deaths of "Protected" Indians, entering under special conditions, which are systematically registered, and of 25 per cent. of the total Indians in the Colony, deaths are rarely registered.

a diagnosis of Malarial Fever would have been impossible. On blood examination the majority of these cases showed either malarial parasites (mostly benign tertian), or mononuclear leucocytosis (large mononuclear leucocytes over 16 %). Later, as the epidemic gathered force, the clinical symptoms increased in intensity, and severe tertian or quotidian agues became common.

Arranged according to notifications (see Chart, p. 462), the number of cases in February was small; the epidemic curve rose sharply early in March, and then with increasing rapidity to reach a fastigium at the end of April—920 cases being notified in the week ending April 29th. Beginning in the second week in May the number of cases rapidly diminished in this and the succeeding month.

The most southerly point from which notifications were received was Umzinto—latitude 29° 44′ S. The commencement of the epidemic, if the earlier cases of ephemeral fever are reckoned, was practically synchronous along the area of coast infected. The distribution of cases was at first patchy, and, although later the disease became fairly general, one district immediately south of Durban appeared throughout to enjoy a relative immunity.

The initial local outbreaks occurred mostly among persons resident along rivers, where the river-beds widen out in broader valleys. It was not until April that malaria crept as it were up the track of the smaller tributary streams. This was due no doubt to the heavy rainfall on the higher ground observed in February, which increased the area of water, and the number of sheltered pools in which *Anophelinae* subsequently bred. In many Indian villages nearly the whole population suffered at one time or another, and the death-roll grew to serious proportions. At the end of April and in May, four outbreaks were reported inland up river valleys as far as thirty miles from the coast. Three of these outbreaks were among Natives, who form almost the entire population in those parts. These outbreaks were traceable to men who had recently been at work near the coast, and had returned home ill. The people became alarmed, and reported that a fatal disease was attacking them. In two instances, the District Medical Officers sent in to the Department of Public Health blood films from persons whom they found actually suffering from fever. Malarial parasites were demonstrated in the films. As many as a score of deaths in one small area were attributed to malaria.

The type of fever has generally been benign tertian, but malignant, or sub-tertian, formed a substantial percentage. In the Government

Hospital, Durban, where over 600 cases were observed, the percentages of each type of infection were determined approximately as follows :—

	Malignant %	Tertian %
White races	20	80
Natives and Indians	8·5	91·5

The apparent racial difference may be due to the different sources of the original mosquito infection; persons infected in various parts of the world constantly arriving in the town of Durban.

In 100 cases of malaria in Indians in country places, we found malignant parasites in 14·4 per cent., the majority towards the end of the epidemic. In the remainder the benign tertian parasite was found. Since no microscopic examination was made in fatal cases the malignant tertian parasite may have been more common. The thin and oily nature of the blood of many Indian patients towards the end of the epidemic, especially in the sub-tertian cases, was very marked.

The causes of the epidemic cannot be determined with any precision for want of accurate data. Since the connection of the mosquito with the life cycle of the malarial parasite has been established, it is evident that the appearance of malaria in a country or region previously exempt from it, may be attributable (*a*) to the introduction of the requisite species of mosquito, not previously existent in the region, (*b*) to the introduction of the parasite in the persons of human beings infected elsewhere, or to both (*a*) and (*b*) together. The occurrence of an epidemic in a region where the disease is sporadic, though indigenous, would be in the main due to the presence of an increased number of suitable mosquitoes, and this again in general would be the result of increased facility for breeding, owing to a difference in the amount and character of the rainfall. Without reverting to the question of the possible existence of endemic malaria in Natal prior to 1905 (see p. 470) it is reasonable to suppose that a number of persons (12,000 or more) imported annually from various parts of India, would harbour malarial parasites capable of infecting suitable mosquitoes. Few cases of malarial relapse have however been recorded in Indians.

Granting that malaria was endemic prior to 1905 then it necessarily follows that suitable mosquitoes must likewise have existed in Natal. Christophers and Stephens[1] have brought forward evidence indicating

[1] *The Practical Study of Malaria.* London, 1904.

that all of the *Anophelinae* are not equally good hosts for malarial parasites and that some species may not act as hosts at all. They believe that the relative prevalence of the disease may depend on the species present in corresponding regions. The Natal epidemic may have been due to the introduction and successful establishment of an Anopheles more favourable to the sexual phase of the protozoon than those previously present. The epidemic may also have been due to a greater number of infected persons recently entering the country, thus increasing the chances of infection in the indigenous species of mosquitoes. The season may moreover have especially favoured the breeding and multiplication of these mosquitoes. Any or all of these hypothetical causes may have operated in this instance.

Mosquitoes found.

From the first, attention was directed to ascertaining what species of *Anophelinae* were principally active in transmitting the disease, but owing to limited time and opportunity and the difficulty in obtaining any material assistance in the collection of live mosquitoes, much less was done than was desired. The majority of those examined were captured by ourselves, and, seeing that all were taken in the daytime, our collection was very probably not representative. We confined our search to habitations in or near which cases of fever had recently occurred, and it is somewhat significant that of more than one hundred and fifty captured, all with two exceptions corresponded, in respect at least of palpal bandings, costal spots, and leg markings, viewed through a hand lens, with Theobald's description of *Pyretophorus costalis*. The exceptions were *Myzorhynchus*, of which the last two and a half hind tarsi were white. No sporozoites were found in the salivary glands of one of the latter examined.

Satisfactory dissection was made of ninety-one of the specimens of *P. costalis*. Eleven of these were brought to us from a house in Durban in which no cases had occurred, although in the vicinity several were reported, and in none of them were sporozoites found. Eighty specimens, for the most part filled with blood, were captured in habitations in the country wherein cases had recently occurred, or wherein persons were at the time lying sick, and sporozoites were demonstrated in thirteen, or 16·2 per cent. (see Plate XV). This cannot be regarded as the actual "sporozoite rate," because many of the insects were kept alive as long as possible after capture, under the most favourable conditions which we were able to devise (see further under *Sporozoites*, p. 482).

As to the part played by any other species of mosquito under natural conditions, we are unable to bring forward any evidence, and opportunity for feeding experiments was not found. The evidence certainly points to *Pyretophorus costalis* as the chief agent.

There is no clear evidence regarding the recent introduction of an increased number of infected persons from India into the country. It is worthy of note that it was found on enquiry that during the previous year a certain number of persons had been drawn from the Malabar Coast, which had not previously been the case. We have some reason to believe that the rate of malarial endemicity is higher on the Malabar Coast than it is in the parts from which the majority of such immigrants come. We have however no evidence that any such persons were infected, and practically no malaria has been reported in any since their arrival. The distribution of these immigrants was investigated, and it was found that in parts of the coast districts to which 72 per cent. of all persons, and 66 per cent. of the children were allotted, malarial fever in this year was severely epidemic and in some places almost pandemic. On the other hand in parts to which 28 and 34 per cent. respectively were assigned, very few cases were notified.

It may be that the opening of the railway line to the area of high endemicity in Zululand has caused a greater influx of Native infected persons to southern parts. For reasons previously stated, there is no reliable evidence as to sickness among Natives. Assuming that Natives from Zululand accounted for the occurrence of malaria in the town of Durban where the majority of such Natives would obtain work, this would not account for the outbreak in country districts where very few, if any, Natives from Zululand are employed. Any hypothesis to be entertained must be capable of accounting for a widespread epidemic commencing synchronously at many different centres separated by considerable distances. For this reason, any suggestion that Chinese immigrants destined for the Transvaal mines were in any way the cause can be immediately rejected. A few have suffered from recurrences of malarial fever after arrival, but the situation of their Depot, by its distance and isolation from any considerable population, precludes the possibility of these having been the origin of the epidemic in the town of Durban, even without considering for the moment more distant places.

With regard to the possibility of the recent introduction of *Pyretophorus costalis* or other *Anophelina* into Natal no judgment can be arrived at for the reason that our knowledge of the mosquitoes of this

country is very scanty. Systematic observations have only been made of recent years, and in certain limited areas, by Mr H. S. Power. It is however worthy of note, that in considering the origin of the epidemic in Mauritius in the late sixties, Giles concludes that the rapidity of extension precludes the supposition that it is attributable to the introduction of a new mosquito. It has been contended that a malaria-bearing Anopheles has been recently introduced into Natal from northern Zululand, where malaria among Natives is severe in parts, especially near the coast. It has been further stated that no *Anophelinae* existed in Durban, until after the railway had been carried north of the Umhlatusi River (latitude 28° 45′ S.). The observations made in this respect have, however, been neither numerous nor systematic, and although occasional search for larvae has not been successful, it may well be that the actual breeding places of the mosquitoes have been missed. We, for instance, have on more than one occasion failed to find larvae where fever was epidemic. The date of opening the railway for traffic from Umhlatusi River (latitude 28° 45′ S.) was July, 1902, but construction trains had been running for some time previous.

We are greatly indebted to Mr H. S. Power, a keen and painstaking observer, for the following information regarding the *Anophelinae* found :—

Year	District	Malaria	Genus and Species
1903	Zululand, 80 miles from rail	Endemic and severe	Pyretophorus cinereus. ,, pitchfordi. Myzorhynchus mauritianus. Cellia squamosa. Nyssorhynchus pretoriensis. ,, maculipalpis. Myzomyia rhodesiensis. ,, funesta.
December	Illovo, 45 miles from coast, 25 miles from rail. Altitude 2300 feet	Not recognised	Pyretophorus costalis.
1903—1904	Inanda, 6 miles from Durban on coast.	Not recognised	Pyretophorus costalis. ,, cinereus. ,, new species. Myzorhynchus mauritianus. ,, paludis Myzomyia funesta.

In nearly all these mosquitoes classification was confirmed by Theobald or Giles.

It is rather remarkable that in careful observations carried over several months, in a part of Zululand having a bad reputation among Natives for fever, the *Pyretophorus costalis* was not found. Anopheles were found many miles to the south of Durban by Mr Power in 1901, but were not classified, and specimens of these are not now available.

In the year 1905, careful and systematic search was made for mosquitoes, and the following *Anophelinae* were identified, captured as imagines, or bred from larvae :—

District	Malarial prevalence	Genus and Species
Coast	Epidemic	Pyretophorus cinereus. ,, costalis. Cellia squamosa. Myzorhynchus mauritianus.
Inland 30 miles; deep valley	2 per cent.; small village	Cellia squamosa. *Pyretophorus cinereus.
Inland 45 miles	Doubtful, probably among Natives	Pyretophorus costalis.
Pietermaritzburg, 45 miles from coast, 2200 feet elevation	Absent	*Pyretophorus cinereus. Nyssorhynchus pretoriensis. Myzorhynchus mauritianus.

* There was some considerable variation in the markings of different specimens, and it is possible that this includes another species. The differences are not yet fully worked out.

We are indebted to Mr C. Fuller, Government Entomologist, for time and trouble spent in examining mosquitoes, and to Mr H. S. Power for information as to the presence of *Pyretophorus cinereus* on the coast, where it was not found by us, and of *Pyretophorus costalis* in an inland district.

From the incomplete knowledge of Anophelinae of different parts of the country, no definite statement can be made, as to whether any particular species was previously present in the area of recognised endemicity, and absent from other areas. Attention may again however be drawn to the observations on *Pyretophorus costalis* as the apparent principal agent in the epidemic, and its absence from a severely affected area in Zululand. It is also worthy of note that this mosquito was found more than a year before cases began to be notified, in an inland district, to reach which from Zululand a change of trains at Durban and a journey by road of certainly not less than twenty-five miles is neces-

sary. In view of the fact that only *P. costalis* was found by us in houses, it seems very improbable that any other species has been the chief agent. It is probable that any new species introduced would become gradually established, at first in some one or two places, and, having become infected, it would at first make its influence felt locally, and not at once over a wide tract of country. The view that newly introduced Anopheles were the agents in this epidemic appears to us scarcely tenable.

If Anopheles were not introduced and there was no notable increase in the number of malarial subjects entering the district during the year 1904 to what was the epidemic in 1905 due? It appears to us that there is but one hypothesis, which, if tenable, would satisfactorily explain the matter; namely a very marked increase in the prevalence of one or more species of *Anophelinae* in the present year, with perhaps somewhat greater opportunity for their infection, by reason of the advent in recent months of somewhat larger numbers of persons harbouring the malarial parasites in latent form. It is worthy of mention that it has been a matter of common repute that "mosquitoes" have been unusually prevalent in the past summer.

In respect of other insects than mosquitoes, great variations in prevalence are observed from year to year, without any assignable cause. It cannot be claimed that meteorological conditions have been especially favourable for mosquito larvae in the past summer.

Observations on Anophelina.

From the beginning of April to the end of June mosquito larvae were collected from a small stream about 18 inches wide running through private grounds belonging to one of us and situated outside Pietermaritzburg. *Nyssorhynchus pretoriensis* predominated in April, but was not found after this month until the end of June, when it was again met with. *Pyretophorus cinereus* was found throughout the period mentioned, but larvae of *Myzorhynchus* were not found, although several imagines were captured at the end of May on the banks of the stream, one being caught in the house. Neither flood nor frost (lowest temperature 30° F.) appeared to greatly affect the number of larvae, although fewer were found in June than in the early part of May. The stream is not more than one hundred yards from the house, but though careful watch was kept, only about one dozen Anopheles were seen indoors, principally in May.

When a visit was made to an inland village to investigate a report of malaria—five cases had occurred—a few pupae and numerous larvae were collected from rock pools and the edges of the river. All the pupae, and 6—8 larvae which developed into imagines in the first ten days after collection, proved to be *Cellia squamosa.* Afterwards, *Pyretophorus cinereus* alone developed, the last hatching out on June 22nd. *Pyretophorus costalis* was not found here, though it may have been present earlier in the year.

Larvae were obtained from the following situations:

Genus and Species	
Pyretophorus costalis	Roadside puddles; cattle footmarks; edge of small marshes; shallow running streams of slow current.
Pyretophorus cinereus	Eddies; under overhanging banks of streams; residual pools.
Cellia squamosa	Residual pools.
Myzorhynchus mauritianus	Pools; dams; edges of small marshes.
Nyssorhynchus pretoriensis	Eddies; pools in streams, and under overhanging banks of faster running brooks; frequently with Pyretophorus cinereus.

Sporozoites.

At first we examined the mosquitoes as soon after capture as convenient, but in the first thirty so examined sporozoites were only found once. It was then decided to keep the captive mosquitoes as long as possible, and dissect such as died, or became very weakly, from day to day. Sporozoites were demonstrated in the salivary glands of 12 per cent. of those examined within the first seven days, and in 30 per cent. of those kept for a longer period[1].

[1] The most suitable diet appears to be sliced apple, with water available for drinking. Apple is preferable to banana, which is apt to entangle the legs. *Pyretophorus costalis* if left undisturbed in the dark thrives well enough in captivity, and feeds freely on human blood if opportunity is afforded; females in captivity occasionally deposited eggs in the drinking troughs, and the eggs hatched out in about forty hours at laboratory temperature in April. The food which suited the larvae best appeared to be dry lean meat, pulverised.

Table showing relative numbers of Pyretophorus costalis examined in which sporozoites were and were not found.

Number of days after capture before examination	Number examined	Sporozoites found	No Sporozoites found
2 and under	44	4	40
3	6	0	6
4	2	0	2
5	6	1	5
6	1	1	0
7	5	2	3
9	8	2	6
10	3	1	2
11	4	2	2
14	1	0	1
Total	80	13	67

This table only shows those which were captured in habitations in which malarial fever had recently occurred.

The method of displaying the salivary glands, attached to the head, by gentle traction on the nape of the neck while the thorax is held stationary, seems only to be satisfactory in mosquitoes just killed. If they have lain some little time, either dry, or in salt solution, the glands appear to become friable, and need to be expressed or dissected out from the thorax. The appearance of the glands when copiously infected differs considerably, even under the half-inch objective, from healthy glands, in that the former are less transparent and rather sticky. In making permanent stained preparations, the method described by Christophers and Stephens of drawing the coverslip along the slide, leaves the sporozoites rather widely scattered. We were able to obtain fuller fields for photographic purposes, by gently lifting the coverslip with a needle, and allowing the salt solution containing the gland tissue to accumulate in a big drop, and then dry. Leishman's stain, about nine days old, gave the best results.

Larvicidal Measures.

In the town of Durban, vigorous action was taken continuously from the end of February by the Medical Officer of Health, in the direction of clearing away vegetation, and the application of kerosene and other materials to all accessible pools, streams, and accumulations of water; but the weekly number of cases continued to increase till the end of April. It may be that *Anophelinae* were breeding in the less accessible parts of the larger swamp, but seeing that this is on the

outside of the town, it is difficult to understand why mosquitoes should travel from there to the centre, in which the natural breeding-places lent themselves well to the successful application of such measures. The water of the harbour abutting on the town contains probably too high a proportion of sea-water to serve as a breeding spot, and there are few rain-water tanks, the town having an abundant water supply. Possibly there were other breeding-places which were not detected, or the duration of life of the average imago is longer than is generally supposed. In the country districts, there were neither legal powers for enforcing necessary measures, nor any adequate staff to carry them out if there had been, and all that could be done was to issue leaflets of advice, and in some instances give more detailed recommendations to sugar planters and other employers of coloured labour. Partly from apathy, and partly from lack of intelligent appreciation of the necessity of attending to minutiae, little was effected, but from observation of the varied character of the water in which larvae were gathered, the prospects of success, where communities are small and water abundant, would not appear very hopeful. In some places the barracks and huts occupied by Indian labourers are undoubtedly much exposed to danger by reason of their proximity to water; in other places they are situated on rising ground, some four or five hundred yards from the nearest water. This of course is well within the flight of mosquitoes, and it would appear advisable to place habitations at a greater distance, but difficulties arise, in that it is repugnant to Indians to dwell, when they can avoid it, otherwise than near to a water supply. It can be readily understood that at the end of a day's work no man wishes to convey his water for domestic purposes from a distance of a mile or so. The provision of mosquito-proof dwellings for these people is clearly impracticable. Under these circumstances only the prophylactic use of quinine appears to give promise.

EXPLANATION OF PLATE XV.

PHOTOMICROGRAPHS.

No. 1. × about 500. Sporozoites free of gland.

No. 2. × about 1000. Sporozoites issuing from cells of salivary gland. In this specimen the protoplasm of the organism stained very faintly, in consequence of which only the chromatin nucleus is shown in the photograph.

Nos. 3 and 4. × about 1000. Sporozoites free of gland substance.

All specimens stained with Leishman's modification of Romanowsky stain.

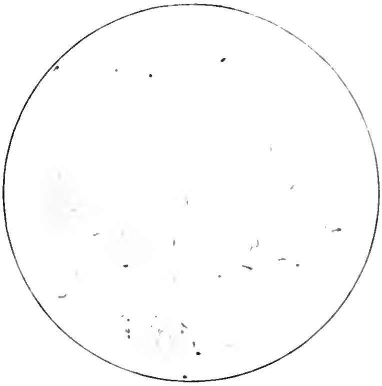


Fig. 1.

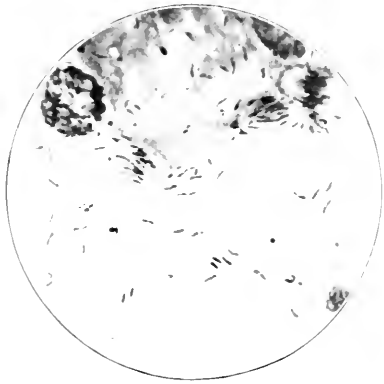


Fig. 2.

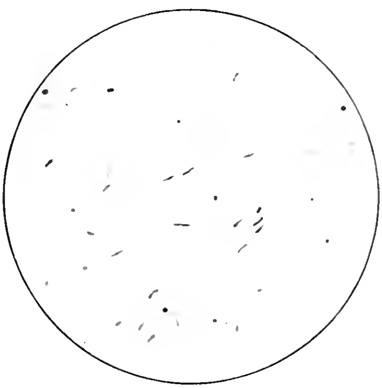


Fig. 3.

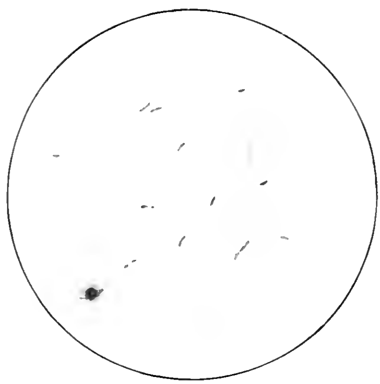


Fig. 4.

NOTE ON THE PREVALENCE OF ANOPHELES.

By GEORGE H. F. NUTTALL, F.R.S.

In a previous paper[1] a record of the distribution of Anopheles in Great Britain was given, the localities mentioned including Cambridge and the surrounding country. Since 1901, Anopheles imagines have been searched for in cellars and houses during the winter, in places where they were known to congregate. During the summer a yearly search for larvae was made in various collections of water in the vicinity of Cambridge with a view to securing material for purposes of study and instruction. It appears worthy of note that there has been a notable decrease in the number of imagines caught in cellars, etc. in the winter months. The insects were fairly numerous in my own house during 1901–1902, as many as 60–100 being found hibernating in the cellar. In 1903–1904 there were fewer, only about 6 being caught. Not a single imagine has been caught in the house since that winter. Whereas Anopheles were frequently caught in houses during 1901–1903 in the warmer months, very few were encountered in 1904, and I have not found a single specimen this year. The common Anopheles in this region is *A. maculipennis*, as I have already recorded, *A. bifurcatus* being less frequently encountered. Apparently corresponding results have to be recorded with regard to Anopheles' larvae. These were certainly very numerous during the period 1900–1903, and they have become scarcer since, being apparently quite absent in certain waters where they formerly abounded. That the Anopheles are not extinguished is certain, for I have found a few larvae (*A. maculipennis*) in the river Cam this summer, and my friend Mr Pearce has brought me an imago from Grantchester (close to Cambridge), in addition to larvae of *A. bifurcatus* which must have hibernated there. Hibernating larvae of this species have twice been found by me since I first recorded their presence about Cambridge.

[1] "Studies in Relation to Malaria," this *Journal*, vol. I, pp. 4—44, 1901.

County	Place	Height above sea in feet	Species of Anopheles found	Notes	Collector
Lincolnshire	Cadney near Brigg	– 100	mac.	Caught one ♀ in his house "on a cold, raw day" with 5 Culex.	E. A. Woodruffe-Peacock 23. III. 1901
Huntingdon	Haughton to St Ives	About 25	mac.	Larvae plentiful along river banks all the way.	G. H. F. N. 5. VII. 1901
	Ramsay	– 50	mac.	Larvae very plentiful in ditch near Abbey, water containing Spirogyra and Lemna. In ditch 1 mile distant on fen, few larvae, water not so clear.	T. S. Pigg 3. VIII. 1901
Cambridge	Grantchester	50—100	mac. bif.	Imago caught in house. Larvae caught in tub.	N. D. F. Pearce IV. 1905
W. Sussex	Slinfold (4 miles W. of Horsham)	– 100	mac.	Larvae found in most streams of district, moderate numbers, none in main river.	Stanley Child 3. IX. 1901
Cheshire	Rowton and vicinity Waverton Saighton Bruera Hatton Heath Christleton Stanford Bridge Tarvin	All 50—100 ,, ,, ,, ,, ,, ,,	mac.	Larvae found in 1 ditch, 1 dried stream, 9 ponds. Of 16 other places tried without result, there were: 7 ponds (Lemna covered), 1 pond (swarmed with tadpoles), 4 ponds very thickly weed-covered, 1 pond very dirty. Found with Culex in 2 ponds, with fish in 3 ponds. Larvae plentiful. 2 imagines caught in house at Rowton Hall. (No cases of locally acquired ague admitted to Chester Infirmary since 1891, no access to older records.)	J. W. S. Macfie 21—28. VI. 1901 10 IV. 1901
Wales **Carnarvon**	Conway	– 50	bif.	Larvae found.	C. W. Daniels IV. 1901
Monmouthshire	Flat country near Major & Redwick round the coast between Chepstow & Newport (nearer Newport)	Near sea level	?	Larvae found. (Well known that there was much malaria thereabouts about 1860—70. No cases now as far as could be ascertained.)	J. Cropper IX. 1901
	At Chepstow		mac.	Imagine caught in house.	,,
Ireland **Galway**	Clonbrock near Ahascragh	?	bif.	One ♂ caught in outhouse.	G. H. F. N. 7. V. 1901

Of course this is but an isolated observation, and necessarily it can have but limited value. It is known of other insects that they are more numerous in some years than in others. For instance *Culex pipiens* abounded in many parts of Cambridge last year, so much so that many persons spoke to me of it, and I captured large numbers in cellars where Anopheles had previously been frequent. In one cellar, in the winter of 1903–1904, I only succeeded in catching one *A. maculipennis*, whilst *C. pipiens* were present in hundreds. We do not know the laws that govern these variations in the number of insects in different years, but it is obvious that variations do occur. It is possible that the reduction in the number of Anopheles in these parts has been due to repeated sudden rainfalls during the last three summers, personally I am strongly inclined to the opinion that these rainfalls have exerted an influence.

Sudden rainfalls would necessarily wash away the larvae in large numbers from the situations which they normally inhabit. It is dangerous to argue from an isolated instance, but knowing that ague formerly prevailed in these parts an explanation of its disappearance may possibly be found in a reduction of the Anopheles persisting for a sufficient length of time to break the chain of parasitism of the malarial parasites in man and the mosquito. It appears again warranted to suggest the desirability of further observations being made along these lines elsewhere.

The preceding data may be added to those already published regarding the geographical distribution of Anopheles in Great Britain[1], the same form of tabulation being adopted here.

I am much indebted to the gentlemen mentioned in the foregoing table for kindly supplying me with the data therein contained. I determined all the specimens collected with the exception of the larvae found by Drs Daniels and Cropper, and the imago of *A. maculipennis* caught by the latter. Both of these gentlemen are well-known as experienced collectors of mosquitoes. I shall be indebted to any gentlemen who will kindly supply me with further facts regarding the geographical distribution of Anopheles in Great Britain.

[1] This *Journal*, vol. I, p. 14 and Maps.

THE CHEMICAL ACTION ON GLUCOSE OF THE LACTOSE-FERMENTING ORGANISMS OF FAECES.

By ARTHUR HARDEN.

(From the Chemical Department, Lister Institute of Preventive Medicine.)

In a former series of experiments I found (1901) that *B. coli communis* attacks glucose in a very characteristic manner, each molecular proportion of the sugar yielding half a molecular proportion of acetic acid and of alcohol, and one molecular proportion of lactic acid, together with a small amount of succinic acid, and gaseous carbon dioxide and hydrogen. Although the amounts of the various substances produced vary somewhat with different organisms the molecular ratio of acetic acid to alcohol remains fairly constant and usually approximates to unity.

MacConkey (1905) has recently observed that the lactose-fermenting organisms of the intestine may be subdivided into four groups according to their varying action on cane-sugar and dulcite, and it was with the object of ascertaining whether these differences are correlated with any difference in the mode of attack of the organisms upon glucose that the following experiments were made.

Experimental Method. The organisms examined had all been isolated by MacConkey (1905) and the methods employed for this purpose will be found described in his paper. They were derived from ten separate samples of normal human faeces (N. H. F.), two of milk, four of water, and one each of cow-dung and horse-dung.

Each organism was cultivated in an atmosphere of nitrogen in 500 c.c. of glucose-peptone-water, containing 10 grammes of glucose, 5 grammes of Witte's peptone, and 5 grammes of chalk, for a fortnight at 37° C. The liquid was then filtered from the insoluble residue and the following analyses made.

1. The residual sugar was estimated by Pavy's method.

2. The total acid produced was estimated from the amount of calcium salt in solution, a correction being applied for dissolved calcium carbonate and calcium in the peptone.

3. Alcohol and acetic acid. The liquid was acidified with excess of oxalic acid, filtered and distilled. The first half of the distillate was collected separately, neutralised by normal caustic soda solution and again distilled, the alcohol being estimated in the distillate by the gravity method.

The total amount of volatile acids was ascertained by titrating the whole distillate, the distillation being continued in a current of steam until 100 c.c. of it only required 0·2 c.c. of normal alkali.

Formic acid, which is usually produced in small amount, was then estimated in the neutralised and evaporated solution by the mercuric chloride method, and the difference between this and the total volatile acids was taken as acetic acid.

4. The non-volatile acids were obtained from the difference between the volatile acids and the total acids produced.

5. The residue from the steam distillation was extracted with ether, the ether evaporated and the residue converted into the zinc salt, the direction of rotation of the plane of polarised light by which was then qualitatively observed.

In all 56 distinct organisms were examined and the results are embodied in the following tables. The groups into which they are divided correspond with the groups 1, 2, 3, and 4 (1) of MacConkey's provisional classification[1].

In addition, the laboratory cultures of *B. coli communis* (Escherich), *B. acidi lactici* (Hueppe), *B. lactis aerogenes* (Escherich), and *B. cloacae* (Jordan) were examined in a similar manner and the results are appended to the tables.

The sugar used is expressed in grammes; the acetic acid and alcohol in molecular proportions per molecule of sugar, and the non-volatile acids (lactic and succinic) in c.c. of normal acid per gramme of sugar.

The rotation of the zinc salt was in every case found to be positive.

[1] *Loc. cit.* p. 352.

No.	Origin	Cane-sugar	Dulcite	Sugar used	Acetic acid	Alcohol	Molecular ratio alcohol / acetic acid	Non-volatile acids
				GROUP 1.				
19	N. H. F. i	–	–	3·78	0·43	0·61	1·43	5·79
20	,, i	–	–	3·41	0·54	0·70	1·29	5·66
21	,, ii	–	–	6·67	0·62	0·57	0·92	5·94
22	,, ii	–	–	6·65	0·65	0·63	0·96	3·79
23	,, ii	–	–	4·24	0·45	0·70	1·57	2·41
24	,, x	–	–	6·84	0·60	0·57	0·95	4·93
25	,, iii	–	–	5·85	0·68	0·66	0·98	2·86
26	,, iii	–	–	5·68	0·50	0·73	1·46	3·88
27	,, iv	–	–	4·76	0·71	0·77	1·09	2·71
28	,, iv	–	–	6·35	0·51	0·51	1·00	4·38
				GROUP 2.				
8	N. H. F. i	–	+	4·05	0·49	0·61	1·24	4·40
9	,, iii	–	+	5·1	0·77	0·72	0·93	2·33
10	,, iv	–	+	5·64	0·66	0·55	0·84	3·83
11	,, iv	–	+	6·13	0·55	0·55	1·00	4·65
12	,, iv	–	+	5·55	0·59	0·50	0·84	4·75
13	,, iv	–	+	5·55	0·57	0·51	0·90	4·73
14	,, iv	–	+	4·36	0·77	0·75	0·98	2·93
15	,, iv	–	+	4·78	0·82	0·75	0·93	3·89
16	,, iv	–	+	6·06	0·56	0·51	0·93	5·11
17	,, iv	–	+	5·82	0·37	0·46	0·81	6·81
18	,, iv	–	+	4·00	0·88	0·76	0·87	4·50
				GROUP 3.				
1	N. H. F. i	+	+	5·02	0·56	0·62	1·21	5·15
2	,, i	+	+	6·15	0·64	0·56	0·87	4·07
3	,, ii	+	+	5·59	0·71	0·71	1·00	3·02
4	,, ii	+	+	3·74	0·86	0·66	0·77	2·46
5	,, viii	+	+	4·6	0·89	0·73	0·82	2·94
6	,, viii	+	+	4·26	0·77	0·60	0·77	5·12
7	,, ix	+	+	4·81	0·75	0·53	0·71	4·16
				GROUP 4. SUB-GROUP 1.				
29	Milk I	+	–	5·6	0·59	0·54	0·90	4·0
30	,,	+	–	5·00	0·60	0·59	0·98	4·85
31	,,	+	–	5·51	0·56	0·55	0·98	4·67
32	N. H. F. vi	+	–	6·21	0·81	0·73	0·90	2·23
33	,, vi	+	–	6·00	0·77	0·62	0·81	3·44
34	,, vi	+	–	6·21	0·54	0·60	1·11	3·73
35	Milk II	+	–	7·2	0·67	0·61	0·91	3·32
36	,,	+	–	6·82	0·72	0·65	0·91	3·17
37	,,	+	–	6·72	0·76	0·67	0·88	2·71
38	Horse-Dung	+	–	6·44	0·69	0·70	1·01	3·39
39	,,	+	–	6·25	0·51	0·53	1·04	5·19
40	N. H. F. vii	+	–	6·87	0·61	0·59	0·98	3·70

Group 4. Sub-groups 2 and 3.

No.	Origin	Cane-sugar	Dulcite	Sugar used	Acetic acid	Alcohol	Molecular ratio alcohol / acetic acid	Non-volatile acids
41	N. H. F. ii	+	–	9·52	0·05	0·77	13·4	1·20
42	,, iii	+	–	10	0·25	0·73	2·89	1·11
43	,, iii	+	–	10	0·27	0·80	2·93	1·32
44	,, iii	+	–	10	0·24	0·74	3·0	1·39
45	,, v	+	–	10	0·12	0·66	5·5	2·17
46	,, v	+	–	10	0·11	0·64	5·8	2·26
47	,, v	+	–	10	0·10	0·65	6·3	2·14
48	,, v	+	–	9·08	0·10	0·59	5·9	1·52
49	Water I	+	–	10	0·03	0·61	18	1·54
50	,, I	+	–	10	0·13	0·69	5·2	0·19
51	,, I	+	–	10	0·05	0·56	11·3	1·87
52	Water II	+	–	10	0·03	0·65	19	0·84
53	Cow-Dung	+	–	10	0·21	0·62	2·94	1·14
54	Water III	+	–	9·12	0·04	0·70	15·5	1·01
55	,, III	+	–	9·12	0·04	0·71	15·8	0·95
56	,, IV	+	–	6·13	0·12	0·65	5·11	0·77

Laboratory Cultures.

57	*B. coli com.* (Escherich)	–	+	5·57	0·58	0·64	1·12	3·67
58	*B. acidi lactici* (Hueppe)	–	–	6·37	0·54	0·51	1·0	5·29
59	*B. lactis aerogenes*	+	–	10	0·14	0·72	5·1	1·00
60	*B. cloacae*	+	–	10	0·05	0·65	13·4	0·85

As might be expected from the nature of the experiments a moderate degree of variation is found between the action of the different organisms classed here in the same group. The members of groups 1, 2, 3, and 4 (1), agree, however, in producing acetic acid and alcohol in a ratio which is not far from 1 and never exceeds 1·5 or falls below 0·7, whilst in the great majority of cases (31 out of 40) it does not differ from 1 by more than 0·2 in either direction. The average value of this ratio deduced from the 40 distinct organisms examined is 0·97. No distinctive difference capable of forming the basis of a system of classification is to be found between the action of the organisms of these four divisions.

The organisms of group 4 however are very sharply divided by this method into two classes, (*a*) Those which conform to the molecular ratio

alcohol/acetic acid = 1; (*b*) Those for which this ratio becomes considerably greater than 2·5.

These organisms are grouped together under the heading Group 4, Sub-groups 2 and 3, and it will be observed in the first place that the ratio of alcohol/acetic acid is always greater than 2·5, varying from about 3 to 19. In addition to this the amount of sugar used is generally large, the whole available amount being fermented in nearly every case, whilst the organisms of the other groups only use up 40–70 % of it. Finally the amount of non-volatile acids produced is much smaller than that characteristic of the other groups.

These same features are characteristic of *B. lactis aerogenes* and *B. cloacae*, the deviation being in the main due to a diminished production of acetic acid, the amount of alcohol being at the same time slightly greater. These two organisms have already been differentiated from *B. coli communis* by the amount and composition of the gas evolved (Theobald Smith, 1895). *B. lactis aerogenes* in fact appears to act on glucose in a totally different manner from the bacilli of Groups 1, 2, 3, and 4 (1). The exact nature and quantitative relations of the products formed are at present under investigation. In the cases of several of the organisms of this last group (Group 4, Sub-groups 2 and 3) the ratio alcohol/acetic acid rises from 3–5, the value characteristic of *B. lactis aerogenes*, to 11–19. The ratio given by *B. cloacae* is 13·4, and it is probable that some of these organisms belong to this or some allied type, but as a detailed chemical examination of the characteristics of such organisms has not yet been completed no definite statement can at present be made on this point.

Summary.

(1) The lactose-fermenting organisms of faeces may be divided into two groups according to the molecular ratio alcohol/acetic acid produced from glucose under specified conditions.

(*a*) Those which produce alcohol and acetic acid in approximately equal molecular proportions.

(2) Those which produce more than 2·5 molecular proportions of alcohol to 1 of acetic acid.

(3) The members of the first of these groups comprise the Groups 1, 2, 3, and 4 (1) suggested provisionally by MacConkey from the

consideration of the action of these organisms on cane-sugar and dulcite. The members of the second of the groups correspond with Group 4, Sub-groups 2 and 3, of MacConkey's provisional classification.

(4) *B. lactis aerogenes* acts upon glucose in a totally different manner from *B. coli communis* and is therefore to be regarded as a distinct organism.

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THE INFLUENCE OF HIGH AIR TEMPERATURES. No. I.

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The aim of the following investigations was to ascertain the limits within which men can continue to exist normally, and to work, when the air temperature is abnormally high: also to study the abnormal phenomena which are produced when these limits are exceeded.

The subject is one of wide interest, not only in connection with the effects of very warm weather or tropical climates, but also because there are many industrial occupations in which men or women have to work daily in very warm air. My attention was first drawn to the subject in connection with the conditions of work in mines and in the cotton and flax textile industries. There are, however, many other occupations, such as work in the stoke-holds and engine-rooms of steamers, in drawing the ovens used for firing pottery, in the drying of salt, etc., where men are exposed to high temperatures; and the effects of even ordinary warm summer weather in producing heat-stroke, especially among soldiers, are well known.

There are many observations showing that men can remain with impunity in temperatures considerably above the body temperature. In tropical countries, for instance, the shade temperature may, if the air be dry, rise to 120° F. (49° C.) without causing much inconvenience. The experiments made by Blagden and Forsyth and by Dobson in 1775[1] prove definitely that for short periods far higher air temperatures can be borne. These observers found that they could remain for a few minutes in a room at about 250° F. (121° C.) without serious inconvenience or marked rise of body temperature, although beef-steaks exposed in the room at the same time and place could be cooked within

[1] *Philosophical Transactions*, Vol. 65, 1775, pp. 111, 463, 484.

13 minutes. They also made a number of experiments showing that the resistance to high temperatures is connected with the evaporation of moisture from the skin. When the air was moistened, so as to prevent evaporation from the skin, very high temperatures could not be borne. The results of these latter experiments coincide with the common observation that the effects of warm air depend largely on its humidity, and that warm air produces much more serious effects when it is moist than when it is dry. Although observations similar to those of Blagden, and Forsyth have been repeated subsequently, there appears, curiously enough, to be a great lack of information as to the exact limits of the air temperature and humidity which can be borne for considerable periods without serious physiological disturbance. Considering the practical importance of the subject this is somewhat remarkable.

The experiments to be detailed below were carried out partly in Dolcoath Mine, partly in the warm incubating room at the Lister Institute in London, and partly at Oxford in a warmed room at the Physiological Laboratory, and in a Turkish bath. In making them I received much valuable help from Dr A. E. Boycott and Mr C. Gordon Douglas, both of whom also acted as subjects in several experiments, and from Mr R. Arthur Thomas, Manager of Dolcoath Mine.

As the physiological disturbances observed were evidently due chiefly to rise in body temperature the main object aimed at was to observe changes of body temperature. The rectal and mouth temperatures were therefore frequently taken during each experiment, with carefully compared pairs of thermometers, which had also been compared with standard instruments verified at Kew and Charlottenburg.

Normal Limits of Rectal and Mouth Temperature. It is well known that under perfectly normal conditions the body temperature varies very appreciably during the 24 hours, and differs sensibly according as it is taken in the rectum, mouth, axilla, or urine, and according also to various other circumstances[1]. As the rectal temperature, which under ordinary conditions gives the highest readings, undoubtedly gives the truest indications of the internal body temperature, its normal variations may first be referred to.

The recent very careful observations of Pembrey and Nicol[2] give a mean difference in rectal temperature of 2·2° F. (1·25° C.) between the time of maximum and that of minimum temperature, the average maximum

[1] For the literature of this subject up to 1897 see Pembrey, "Animal Heat" in Schäfer's *Text-book of Physiology*, Vol. I, pp. 788, 798.

[2] *Journal of Physiology*, Vol. XXIII, p. 388, 1898.

temperature (about 99·4° F. or 37·45° C.), being in the afternoon between 4 and 7, and the average minimum (about 97·2° F. or 36·2° C.) in the early morning between 2 and 5. These variations cannot, however. be taken as indicating completely the normal limits of temperature variation in healthy persons, since either muscular work or a moderately warm external temperature may cause a rise considerably above the maxima just quoted. Thus after ordinary muscular work, such as walking for about an hour, Pembrey and Nicol, and Pembrey, Arkle, Bolus, and Lecky[1] observed rectal temperatures as high as 101·3 (38·5° C.). On a warm summer afternoon at a temperature of 88·5° F. (or 31·5° C.) Pembrey and I took the urine temperatures of 83 soldiers wearing light khaki uniforms[2]. The average temperature found was 99·65° (37·6° C.) In 19 cases the temperature was 100° (37·8° C.) or more, the maximum (two cases) being 100·8 (38·2° C.). As the men had done no muscular work and were in good condition, and as the urine temperature is usually slightly below that in the rectum, these observations indicate that on a warm day the rectal temperature is commonly very distinctly higher than in cool weather. The experiments detailed below also indicate that the upper limit of what may still be regarded as a normal rectal temperature must not be placed too low, certainly not below 101° F. (38·3° C.), although the same temperature if it occurred in cool weather and without previous muscular work would be distinctly abnormal.

The normal limits of the mouth temperature are certainly wider, as a rule, than those of the rectal temperature. This has recently been pointed out very clearly by Pembrey and his coadjutors in the papers already quoted. They found that the mouth temperature is not a reliable index of the deep temperature, particularly during cold weather or after exercise. They sometimes found that the mouth temperature was as much as 4·5° F. (2·5° C.) below that of the rectum, and that during work the mouth temperature might fall while the rectal temperature was rising considerably. In the course of the experiment described below many instructive observations were made as to the difference between mouth and rectal temperature. In the first place it was found that this difference varies greatly with the individual. In the case of myself the rectal temperature is nearly always, under ordinary conditions, higher by about a degree or a little more than the mouth temperature. In warm and moist air as the rectal temperature rises the two temperatures tend to approximate, but on returning to a moderate temperature they again

[1] *Guy's Hospital Report*, Vol. LVII, 1902, p. 283.

[2] *Ibid.* p. 304.

separate, and may for a time be as much as 3·2° F. (1·8° C.) apart (see exp. I.) In the case of C. G. D., on the other hand, the rectal and mouth temperatures keep much closer together, and are often practically the same, though usually the mouth temperature was found to be slightly lower (see experiments XI to XXII).

The fact that the mouth temperature is frequently a good deal below the body temperature depends evidently on cooling caused by the proximity of the skin, and possibly also of the nasal cavity. In very warm air, where this cooling action disappears, the two temperatures approximate, and we found in accordance with this explanation that when the wet-bulb temperature of the air reached or exceeded the body temperature the mouth temperature might be distinctly higher than the rectal (see experiments X, XI). In all our experiments where the mouth temperature was being observed the mouth was kept closed continuously, so that no fallacy could arise from the cooling action of air passing through the mouth. In many experiments the thermometers had to be kept in continuously except when being read. In very hot air this precaution was necessary, as the thermometers could not have been read and would have very quickly burst if exposed to the heat outside.

Observations in Levant and Dolcoath Mines. My attention was first directed to the need for the present investigation by personal experiences in Levant Mine during an investigation on the causes of phthisis and anaemia among Cornish miners. This rich tin and copper mine runs out for about a mile under the Atlantic in the neighbourhood of St Just. Owing to various difficulties the ventilation is very inadequate, the temperature being correspondingly high, owing largely to accumulation of heat in the mine from the oxidation of iron pyrites. The air is nearly everywhere saturated with moisture, and the temperature of the workings varies from about 80° to 93 (26·7 F. to 33·9° C.[1]). On my first visit to this mine I experienced much discomfort, and my mouth temperature rose, even in the cooler parts of the mine, when the air temperature did not exceed about 86° F. (30° C.), to 102·5° F. (39·2° C.). The mouth temperature of another member of the party (J. S. M.) rose to 101·5° F. At the time we were only walking slowly, and had not climbed any ladders, but were wearing flannel shirts and canvas coats. At a second visit we climbed down 400 feet to the very warm bottom levels, where the

[1] For details as to the temperature and composition of the air in this and other Cornish mines, see Appendix III. to "*Report on the Health of Cornish Mines*," Parliamentary Paper Cd. 2091, 1904, by Haldane, Martin, and Thomas.

temperature was about 92° F. (33·3° C.) in the warmer parts, and afterwards climbed up. I had on only a light cotton shirt, open at the front, and this time my mouth temperature only rose to 100·7° F. (38·2° C.), and I felt no inconvenience apart from the excessive sweating. In the case of J. S. M., who had kept on a flannel shirt and cotton coat, the mouth temperature rose to 102·2° F. and marked discomfort and shortness of breath were produced, making rests at each level necessary. In another member of the party, who was similarly clad to myself, and was an assistant mine manager, the mouth temperature only rose to 99·7° F. (37·6° C.). It is a common thing for visitors to the bottom parts of the mine to be greatly affected by the heat, and consequently experience much difficulty in climbing up again to the bottom of the main shaft[1]. The men who are accustomed to the mine, on the other hand, appear to bear the heat well, and not to suffer in health. They do not, however, seem to be able to do more than a limited amount of work. The

[1] Dr A. E. Boycott has given me the following very interesting account of his experiences when he visited the mine, accompanied by Mr Cadman, H.M. Inspector of Mines, in order to investigate the conditions with reference to the possible presence of ankylostomiasis among the miners:

"We climbed straight down one of the shafts (temperature 78° F. at the bottom) to the 278 fathoms level, and walked out under the sea along the pony road, soon discarding our coats, and going in vests and trousers only. We then climbed down the submarine shaft (temperature 86° wet and dry bulb at the top, and 87° at the bottom) to the 302 fathom level, and walked out westwards about half-a-mile, I should think, to an "end" where the temperature was 93°, air saturated. The going was bad, and I had to stoop most of the way, as the road was largely timbered. When we reached the end we had been underground I daresay three hours, as we had been collecting samples of faeces, etc. I felt very hot, and was glad to sit down. My mouth temperature was 103·5° by an ordinary clinical thermometer which C. read for me. There were a man and a boy in the end supposed to be hand-drilling, but they did not seem to be doing anything except sweating. As you know, the men are reported to wet the drill-holes by pouring the sweat out of their boots! Coming back I did pretty well till we had to come up the ladders from the 302 to the 278. After the first one I made up my mind I should never get up the rest, as severe dyspnoea came on when I had gone a few steps up each ladder. These were, I suppose, about three fathoms each, and I had to lie down for five minutes or so at the top of each one and gasp. When we got up to the pony road and had a drink of water I soon felt pretty well all right and came up the man-engine (my first experience) without trouble except when I lost my light. By the time I had washed and changed I felt quite well, and regretted that we had wasted our opportunities of collecting samples of faeces. After I had been in the end I felt no interest in the matter. I am sure the psychological effect of knowing that there was no gig, and that I had to climb up or face the unknown terrors of the man-engine, had a good deal to do with my feeling beaten. Cadman did not feel it nearly so much as I did. A few weeks later I did three much longer days running in Talke, Birchenwood, and Snailbeach, and though I got tired in the four-feet roads and was very stiff afterwards I never felt at all knocked up."

leisureliness of all work in the mine is in very striking contrast to what may be observed in any ordinary English colliery of about the same depth. Perhaps the most surprising thing was that profitable work could be carried on at all under existing conditions, and that the men appeared to be exceptionally healthy for Cornish miners. Owing to the dampness of the mine they suffered less from inhalation of stone dust than many other Cornish miners; and owing probably to the fact that salt water was everywhere present they were free from ankylostomiasis[1].

These observations suggested the desirability of investigating the effects of various definite air temperatures and degrees of humidity of air on the body temperature under different circumstances of clothing, air-currents, and work: also the physiological effects of rise of body temperature on the respiration, circulation, and other functions, and on the capacity for performing muscular work. The first experiments on these lines were made in Dolcoath Mine, in order to ascertain the effects of motionless saturated air at different temperatures without work, and with a minimum of clothing. A hot level was at the time being driven, along which temperatures varying from 85° to about 98° F. could be selected at times when the ventilation was suspended. The dry and wet bulb thermometers read practically the same at all parts, so that the air was saturated. The observations, which were very instructive, may be quoted in full.

EXPERIMENT No. I.

11.30 a.m., at surface. Rectum temperature, 100·2°; mouth temperature, 99·2°[2].

12·2 p.m. Entered level and remained standing at place where air temperature was 94° dry and 93·6° wet. All clothing removed except boots and stockings, flannel drawers and canvas trousers.

12.15 Sweating profusely. Rectum, 101°; mouth, 99·8°. Took temperatures of two miners who had been in the end mending an air-pipe for nearly an hour, and were coming out to "cool off." In each the mouth temperature was 101·6°.

12.40 Rectum, 101·4°; mouth, 100·8°. No discomfort.

12.45 Urine, 101·5°.

1.0 Some throbbing of head.

1.7 Rectum, 101·6°; mouth, 101·5°.

1.12 Pulse (standing), 138. Walked slowly up and down the level till 1.20.

1.25 Rectum, 102·6°; mouth, 102°. Pulse (standing), 144. Marked throbbing. Breathing seems deeper, and there is a tendency to breathe through the mouth.

[1] Boycott and Haldane, this *Journal*, IV, p. 108, 1904.

[2] 37° C. = 98·6° F.: 1·8° F. = 1° C.

1.45 Rectum, 103·5°; mouth, 102·6°. Pulse (standing), 164; (sitting), 140. Respirations, 20; the breathing being both deeper and more frequent than usual. Much throbbing, and feeling of general discomfort.

1.55 Came out into air-current in a short cross-cut leading to the level. Air temperature, 85·5° dry; 84·5° wet. Clothes as before.

2.10 Rectum, 104·2°; mouth, 102·1°. Feeling better, but head still throbbing.

2.20 Put on flannel shirt and canvas coat, and ascended the shaft in the gig.

2.30 In office near the top of the shaft. Air temperature, 60°; rectum, 103·9°; mouth, 100·7° (10 minutes' observation). Pulse (standing), 124. No hyperpnœa now.

2.42 Rectum, 103·1°; mouth, 100·2°. Pulse (standing), 122; (sitting), 106. Quite comfortable, but still perspiring. Now walked about ¼-mile to main office, and drank some milk and soda.

3.18 Rectum, 101·2°; mouth, 99·6°. Pulse (standing), 112; (sitting), 102. No sweating now, and no discomfort.

3.42 After a tepid bath and dressing. Rectum, 101·1°; mouth, 98·9°. Pulse (standing), 102; (sitting), 102.

5.35 After lunching and returning from the mine. Rectum, 100·1°; mouth, 98·2°; urine, 99·6°. Pulse (sitting), 98.

11.30 Urine, 98·6°; mouth, 97·6°. Pulse (sitting), 84.

9 a.m., next day. Rectum, 98·2°; mouth, 97·6°; urine, 97·9. Pulse (standing), 80; (sitting), 76.

These observations show that the subject of the experiments was unable to maintain a normal body temperature in still and saturated air at 94°. Throbbing in the head and increased frequency of the pulse were the most marked symptoms observed. The throbbing was not perceptible until the rectum and mouth temperatures had reached about 101·5°. It is interesting to note that on coming into cool air the rectal and mouth temperatures differed by as much as 3·2°, although the mouth was kept closed, and the thermometer was left for 10 minutes under the tongue.

As a normal temperature could not be maintained in still and saturated air at 94° (34·4°), a second experiment, at an air-temperature which remained at 89° (31·5°) by both wet and dry bulb, was made in the same level. The clothing was the same as before.

Experiment No. II. 17/4/05.

11.20 a.m., at surface. Rectum, 99·0°; mouth, 98·2°.

12.0 Went into level, and stopped where temperature was 89° (30·7°).

12.10 Sweating profusely. Rectum, 99·9°; mouth, 99·5°.

12.15 Pulse, 102 (standing).

12.40 Rectum, 100·3°; mouth, 99·9°. Slight throbbing in head.

12.45 Pulse, 110 (standing).

1.5 Rectum, 101·2°; mouth, 100·6°.

1.20 Pulse, 130 (standing).
1.35 Rectum, 101·6°; mouth, 101·0°. Throbbing scarcely noticeable.
1.40 Pulse, 120 (standing); 112 (sitting).
1.53 Rectum, 101·9°; mouth, 101·2°. Not sweating so much. Feel all right. Slight throbbing and tendency to breathe through mouth.
2.5 Pulse (standing), 120; (sitting), 112.
2.15 Rectum, 102·4°; mouth, 101·4°.
2.32 Rectum, 102·7°; mouth, 101·5°.
2.47 Rectum, 102·8°; mouth, 102·0°. Pulse, 124 (standing); 112 (sitting). Feeling no worse. Came out and was wound up to surface.
3.15 After walking to office with shirt and coat on. Rectum, 101·5°; mouth, 99·4°.

In this experiment also the temperature gradually rose above normal, and was still rising at the end of $2\frac{3}{4}$ hours. It thus appeared that even 89° (31·7°) in motionless and saturated air was slightly above the limit at which a normal regulation of body temperature occurred. The experiment was prolonged in the expectation that after a slight rise of body temperature a stage of equilibrium would be reached; but this was not the case, since the body temperature was still rising steadily after $2\frac{3}{4}$ hours.

The next six experiments were made in the incubation room at the Lister Institute, London, Dr Boycott and myself being the subjects. This room is heated by hot-water pipes, which maintain a very constant temperature. The dry and wet bulb temperatures were obtained by waving the two thermometers in the air at the level of the nipples until the readings were constant. The wet bulb read about 1° higher if not waved about. The humidity of the air was raised by boiling a kettle in the room, and by leading steam in through a pipe. The first three experiments were somewhat defective owing to the fact that the air in the room was cooler and fresher below. Thus at the knee-level the temperature by both wet and dry bulb was about 10° F. lower. Although our legs and feet were clothed this may have made a difference: hence

EXPERIMENT NO. III. 5/5/05.

Time	Air temperature °F.		Body temperature in °F.				Pulse (sitting)		Remarks
			Rectal		Mouth				
	Wet bulb	Dry bulb	J. S. H.	A. E. B.	J. S. H.	A. E. B.	J. S. H.	A. E. B.	
Just before entering	—	—	99·4	99·9	98·5	99·1	—	—	
20 mins. after entering	78·5	108	99·6	100·3	98·8	99·6	100	93	Sweat dripping from faces of both persons.
50 ,,	80·2	108	99·9	100·2	98·9	99·8	100	90	
80 ,,	83	109	100·0	100·1	98·9	99·8	88	90	Still dripping slightly from face of J. S. H. Rest of skin moist.
100 ,,	84	109	100·2	100·1	99·0	100·1	—	100	

Experiment No. IV. 6/5/05.

Time	Air temp. °F.		Body temp. °F.				Pulse (sitting)		Remarks
			Rectal		Mouth				
	Wet bulb	Dry bulb	J. S. H.	A. E. B.	J. S. H.	A. E. B.	J. S. H.	A. E. B.	
Just before entering	—	—	99·6	98·9	98·3	98·3	—	—	
20 mins. after entering	85	114·5	99·9	99·4	98·7	99·4	90	88	Both sweating profusely.
35 ,,	86	113	—	99·7	—	99·6	—	94	
60 ,,	88	113	100·2	100·3	98·8	99·6	—	104	
80 ,,	87·5	113	100·5	100·3	99·2	99·6	92	100	Feel quite comfortable but for profuse sweating.
105 ,,	87·5	112·5	100·8	100·1	99·4	99·8	95	90	
130 ,,	88·5	112·5	101·2	—	99·7	—	—	—	J. S. H. feels quite comfortable: A. E. B. not comfortable, and had a headache the same evening.

after exp. V a platform was erected, on which we sat; steam being led in below, so that our heads and feet were in nearly the same temperature. Clothing as in the first two experiments.

Experiment No. V. 9/5/05.

Time	Air temp. °F.		Body temp. °F.				Pulse (sitting)		Remarks
			J. S. H.		A. E. B.				
	Wet bulb	Dry bulb	Rectum	Mouth	Rectum	Mouth	J. S. H.	A. E. B.	
Just after entering	—	—	100·2	98·9	100·1	98·3	—	94	
20 mins. after entering	83	101	—	—	100·1	98·8	92	84	Both sweating freely. Pulse of J. S. H. 104 standing.
40 ,,	88	101	100·4	99·1	99·9	99·4	—	87	
60 ,,	88	103	—	—	—	—	—	—	
80 ,,	89	103	100·5	99·4	99·7	99·4	—	94	
100 ,,	89·5	103	—	—	99·9	99·4	93	88	
120 ,,	90	104	100·5	99·5	—	—	—	—	
150 ,,	90	104	101·1	100·1	99·5?	99·8	—	—	No discomfort, apart from sweating.

Experiment No. VI. 12/5/05.

(Platform now erected in the room.)

Time	Air temp. °F.		Body temp. °F.				Pulse (sitting)		Remarks
			J. S. H.		A. E. B.				
	Dry bulb	Wet bulb	Rectum	Mouth	Rectum	Mouth	J. S. H.	A. E. B.	
Just before entering	—	—	99·7	98·7	99·7	98·5	—	—	
20 mins. after entering	97·5	86	99·9	99·0	99·7	99·1	—	84	Sweating freely.
40 ,,	97·5	86	99·8	98·9	99·5	99·0	84	86	
60 ,,	97·5	86	99·9	98·9	99·8	99·2	—	90	
70 ,,	101	89·5	—	—	—	—	—	—	Sweating increased.
80 ,,	101	89·5	—	—	99·8	99·4	100	93	
90 ,,	102	90	100·5	99·5	—	—	104	98	Not feeling so comfortable. Slight throbbing, &c.
100 ,,	103	90·3	100·9	100·0	100·1	99·8	100	110	
120 ,,	103	91	101·1	100·5	100·7	100·6	106	116	A. E. B. felt "slack" for some hours afterwards.

EXPERIMENT NO. VII. 15/5/05.

(A. E. B. in chamber alone.)

Time	Air temp. °F. Dry bulb	Air temp. °F. Wet bulb	Body temp. °F. Rectum	Body temp. °F. Mouth	Pulse (sitting)	Blood-pressure in mm. Hg.	Haemoglobin %	Remarks
Just before entering	102·5	88	99·3	98·5	88	107	105	
20 mins. after entering	103	91	99·2	99·1	92	—	—	Sweating freely.
40 ,,	103	89·5	99·4	99·5	98	—	—	
55 ,,	102·5	90	99·8	99·8	98	106	—	
75 ,,	102·5	90·5	100·3	100·1	112	—	—	Feel all right.
100 ,,	102·5	91	100·7	100·4	114	—	—	Less comfortable.
120 ,,	102	89·5	101·1	100·4	112	—	—	Felt nausea and great discomfort; which disappeared half-an-hour after coming out, except for a slight headache during the evening.
40 mins. after leaving	—	—	—	—	—	—	112	

EXPERIMENT NO. VIII. 19/5/05.

(J. S. H. in chamber alone. Experiment chiefly with a view to observing the effects of rise of body temperature on the alveolar CO_2 %. See Haldane and Priestley, *Journ. of Physiol.* XXXII, p. 225, 1905; and FitzGerald and Haldane, *Ibid.* XXXII, p. 486.)

Time	Air temp. °F. Dry bulb	Air temp. °F. Wet bulb	Body temp. °F. Rectum	Body temp. °F. Mouth	Pulse (sitting)	CO_2 % in dry alveolar air: End of inspiration	CO_2 % in dry alveolar air: End of expiration	Blood-pressure in mm. Hg.	Remarks
Just before entering	—	—	99·7	98·6	—	5·64	5·65	114	
10 mins. after entering	103	86	—	—	88	—	—	—	Sweat dripping freely.
20 ,,	103·5	90	—	—	—	—	—	—	
40 ,,	103	93	100·1	99·7	110	—	—	—	
55 ,,	105	93	100·6	100·1	112	5·42	5·64	—	
80 ,,	105	94	101·7	101·1	118	—	—	—	Slight throbbing. No headache or hyperpnoea.
100 ,,	104	94·5	—	—	—	4·93	4·80	—	
110 ,,	102·5	94	102·9	102·2	132	—	—	—	Throbbing in head. Feel rather uncomfortable.
120 ,,	102·5	93·5	—	—	—	4·64	4·98	—	Decidedly uncomfortable.
140 ,,	103	93	103·8	103·5	140	—	—	132	
150 ,,	102·5	92	—	—	—	4·55	4·79	—	
157 ,,	—	—	—	—	—	—	—	—	Came out.
5 mins. after leaving	68	—	103·9	102·5	124	—	—	—	
40 ,,	68	—	102·5	99·8	110	—	—	—	Still sweating. Had stood in wet pyjamas since coming out.
70 ,,	68	—	101·0	98·4	104	—	—	—	Had changed into ordinary clothes. Standing with coat off. Skin still damp with sweat. Feel all right, very slight headache.
85 ,,	68	—	—	—	—	5·26	5·23	—	Felt slight headache and tired during evening.

Experiment No. IX. 2/6/05.

(This experiment was made with a view to observing the influence of an air-current in modifying the effects of warm air on the body temperature. The subject sat in an air-current produced by an electric fan, other conditions being as in the previous experiments. J. S. H. alone in room.)

Time	Air temp. °F. Dry bulb	Wet bulb	Body temp. °F. Rectum	Mouth	Pulse (sitting)	Remarks
Just before entering	—	—	100·3	99·0	—	Perspiring slightly at the time.
After 5 mins.	100	86·5	—	—	—	
,, 15 ,,	104	91·5	—	—	—	Air current = 170 linear feet per minute. Dripping from face.
,, 25 ,,	102·5	92	100·3	99·4	96	Air velocity At chest level = 190 ft. per min. ,, face ,, 170 ,, ,, ,, knee ,, 130 ,, ,,
,, 40 ,,	104	92·5	—	—	—	
,, 50 ,,	101·5	92	—	—	99	
,, 60 ,,	103·5	93·5	100·5	99·6	102	
,, 75 ,,	102	94	—	—	—	Air velocity = 190 feet per minute at chest level.
,, 90 ,,	100·5	93	100·8	99·9	102	
,, 110 ,,	105	95	—	—	—	
,, 125 ,,	103	94	101·2	99·4	120	Little or no discomfort, except for sweating.
7 mins. after leaving	66	—	—	—	114	

The next three experiments were made in a Turkish bath at Oxford at higher temperatures. The Turkish bath was divided in the usual way into several chambers, heated by a constant current of hot air passing through them and through the air-space in the double walls surrounding the chambers. The air was simply heated by passing through a furnace, and thus contained only the moisture which was present in the outside air. As the experiments were done during warm and rather moist weather at Midsummer the proportion of moisture in the outside air was considerable, the dew-point being about 55° F. (13° C.), corresponding to about 1·5 % by volume of aqueous vapour. The clinical thermometers were kept in position continuously, except when readings were taken, at which times it was necessary to come out for about one minute as the thermometers would otherwise have risen on taking them out. The time lost in this way was deducted in the record. Clothing as in the previous experiments.

Experiment No. X. 20/6/05.

Time	Air temp. °F. Dry bulb	Wet bulb	Body temp. °F. Rectum	Mouth	Pulse (standing)	Remarks
Just before entering	—	—	99·5	98·2	—	
After 3 mins.	182	97·2	—	—	—	Skin already moist.
,, 10 ,,	—	—	—	—	132	Dripping freely.
,, 20 ,,	—	—	101·0	102·3	—	
,, 32 ,,	182	97·2	102·3	102·3	—	Feel uncomfortable. Marked throbbing of heart.
Outside air afterwards	60	55·5	—	—	—	Quite comfortable on coming out.

It should be noted that the wet-bulb thermometer read about 2° higher when left stationary, and that the readings were taken at the level of the nipples. At the face level the readings were a degree or two higher, and at the knee level several degrees lower.

Experiment No. XI. 22/6/05.

Time	Air temp. °F. Dry bulb	Air temp. °F. Wet bulb	Body temp. °F. J.S.H. Rectum	Body temp. °F. J.S.H. Mouth	Body temp. °F. C.G.D. Rectum	Body temp. °F. C.G.D. Mouth	Pulse (standing) J.S.H.	Pulse (standing) C.G.D.	Remarks
Just before entering	—	—	98·9	98·1	99·4	99·0	—	—	
After 1 min.	165	98	—	—	—	—	—	—	Skin moist.
,, 17 mins.	—	—	99·5	99·4	100·1	100·6	114	—	
,, 22 ,,	—	—	—	—	101·4	101·6	—	—	C. G. D. felt faint, and had to leave.
,, 33 ,,	163	98	100·2	100·5	—	—	—	—	
,, 38 ,,	165	98	—	—	—	—	134	—	
,, 53 ,,	—	—	102·8	102·3	—	—	—	—	
2 mins. afterwards	110	—	—	—	—	—	140	—	Pulse 134 sitting.
11 ,,	110	—	103·0	101·8	—	—	—	—	
28 ,,	110	—	102·0	101·0	—	—	—	—	Just after cold douche. Both feel well and comfortable. Pulse of J.S.H. 90 sitting.
70 ,,	75	65	—	—	—	—	—	—	In outside air.

Experiment No. XII. 30/6/05.

Time	Air temp. °F. Dry bulb	Air temp. °F. Wet bulb	Body temp. °F. J.S.H. Rectum	Body temp. °F. J.S.H. Mouth	Body temp. °F. C.G.D. Rectum	Body temp. °F. C.G.D. Mouth	Pulse (standing) J.S.H.	Pulse (standing) C.G.D.	Remarks
Just before entering	—	—	100·1	98·7	100·0	99·4	—	—	
After 5 mins.	135	89	—	—	—	—	101	—	Damp all over and beginning to drip from face.
,, 20 ,,	—	—	100·1	99·0	100·2	100·3	—	—	Sweating profusely.
,, 38 ,,	136	89	100·2	99·2	100·6	100·4	96	—	
,, 48 ,,	136	89	—	—	—	—	108	—	
,, 57 ,,	—	—	100·6	99·6	101·3	101·0	—	108*	
,, 75 ,,	136	89	100·7	99·9	101·5	101·4	—	—	
,, 85 ,,	—	89	—	—	—	—	114*	109*	
,, 93 ,,	—	—	101·3	100·2	101·7	101·6	—	—	No discomfort.
20 mins. after coming out	—	—	—	—	99·9	99·0	—	—	After a swim in tepid water.
40 ,,	65	62·5	—	—	—	—	—	—	In outside air.

The succeeding experiments, which were at lower temperatures, were made at the Physiological Laboratory, Oxford, with a view, more particularly, to observing the effects of muscular work in warm and moist air. The room used for the experiments was gradually heated up

by keeping gas burning in it continuously, with the windows and door shut. The air of the room was only slightly unpleasant, as the Oxford gas is well purified from sulphur[1], and the proportion of CO_2 did not exceed about 0·3 %. The moisture in the air was further increased, when required, by evaporating water in the room. As C. G. D., who was the subject of several of the experiments, had not been tested during rest at a lower wet-bulb temperature than 89° F. (31·7° C.), experiment No. XIII was made in order to ascertain whether like J. S. H. and A. E. B. his temperature remained about normal when resting, stripped to the waist, at a wet-bulb temperature below 88°.

Experiment No. XIII. 16/7/05.

(C. G. D. as subject.)

	Air temp. °F.		Body temp. °F.	
Time	Dry bulb	Wet bulb	Rectum	Mouth
Before entering	—	—	99·1	99·1
After 20 minutes	97	86·5	99·2	99·3
,, 40 ,,	97	88	99·6	99·6
,, 60 ,,	95	86·5	99·7	99·6
,, 80 ,,	95	86	99·6	99·5

The work in the following experiments was performed by ascending four times a minute a step-ladder standing on the floor of the room. The step-ladder was $3\frac{1}{2}$ feet (1·06 metres) high. It was sloped at a convenient angle, and as it was also provided with a rail for holding on to, it could be ascended and descended very easily. Although this could easily be done regularly six times a minute, an ascent four times a minute was found to be sufficient, and obviated all hurry. As J. S. H. weighed 185 lbs. (84 kilos), and C. G. D. 145 lbs. (66 kilos), the work done per minute in ascending was 2590 foot-pounds (350 kilogrammetres) for J. S. H. and 2030 (276) for C. G. D.

The dry and wet bulb thermometers were fixed at a height of 5 feet. As the wet bulb read too high when in a fixed position a correction (about 1·5°) was made in its readings.

The clinical thermometers were kept *in situ*, and read every 10 minutes, an interval of 1 minute (deducted in the record) being allowed for this purpose.

[1] See this *Journal*, III, p. 382, 1903.

EXPERIMENT NO. XIV. 15/7/05.

(Work. J. S. H. as subject.)

me	Air temp. °F. Dry bulb	Air temp. °F. Wet bulb	Body temp. °F. Rectum	Body temp. °F. Mouth	Remarks
Just before beginning	88·5	78·5	100·6	99·1	Sweating slightly.
After 10 mins'. work	—	—	100·7	99·9	Pouring with sweat.
,, 19 ,,	—	—	101·2	100·1	
,, 28 ,,	—	—	101·5	100·5	
,, 37 ,,	89	78·5	101·6	100·7	
,, 46 ,,	—	—	102·0	101·0	
,, 55 ,,	—	—	102·2	101·1	
,, 64 ,,	—	—	102·5	101·3	
,, 73 ,,	—	—	102·6	101·3	
,, 82 ,,	88·5	79·5	103·0	101·5	Considerable throbbing and dyspnoea. Stopped.
30 mins. after stopping	—	—	102·2	99·5	Had stood in the room with shirt off. Still sweating.
60 ,, ,,	—	—	101·5	99·4	Had stood in the room with shirt off. Sweating stopped.

EXPERIMENT NO. XV. 15/7/05.

(Work. C. G. D. as subject.)

Time	Air temp. °F. Dry bulb	Air temp. °F. Wet bulb	Body temp. °F. Rectum	Body temp. °F. Mouth	Remarks
Just before beginning	—	—	99·7	99·5	
After 10 mins'. work	89	79·5	100·0	100·0	Not yet sweating.
,, 20 ,,	87·5	79·5	100·3	100·3	Sweating.
,, 30 ,,	—	—	100·7	100·7	
,, 40 ,,	86·5	79·5	100·7	100·6	
,, 50 ,,	—	—	100·9	100·6	
,, 60 ,,	87·5	79·0	101·0	100·7	
,, 70 ,,	—	—	101·1	100·7	
,, 80 ,,	88	79·5	101·1	100·7	No discomfort.

EXPERIMENT NO. XVI. 16/7/05.

(Work. J. S. H. as subject.)

Time	Air temp. °F. Dry bulb	Air temp. °F. Wet bulb	Body temp. °F. Rectum	Body temp. °F. Mouth	Remarks
Just before beginning	97	85	99·7	98·8	Feel quite cool.
After 10 mins'. work	—	—	99·8	99·6	Sweating profusely five mins. after starting.
,, 20 ,,	97	86·5	100·2	100·2	
,, 25 ,,	—	—	100·7	100·7	
,, 35 ,,	97·5	88·5	100·9	101·2	
,, 45 ,,	—	—	101·7	101·8	
,, 55 ,,	—	—	102·7	102·4	Much dyspnoea and throbbing. Mouth thermometer removed on account of dyspnoea.
,, 60 ,,	95·5	87	103·1	—	Stopped, much dyspnoea, throbbing, and feeling of exhaustion.
5 mins. after stopping	—	—	103·3	—	Had stood in warm room since stopping. Still some dyspnoea.
30 ,, ,,	70	61	102·3	—	Had stood in cool room for 25 minutes without putting on clothes. Felt "slack" for some hours.

Experiment No. XVII. 20/7/05.

(Work in cool room. Windows and door shut. J. S. H. as subject.)

Time	Air temp. °F.		Body temp. °F.		Remarks
	Dry bulb	Wet bulb	Rectum	Mouth	
Just before beginning	71	63	99·8	98·6	
After 10 mins'. work	—	—	99·8	99·1	Forehead damp. Rest of skin slightly moist.
,, 20 ,,	71	63	100·3	99·5	Some drops on forehead. Rest of skin moist.
,, 30 ,,	71	63·5	100·6	99·5	Slight dripping from face. Rest of skin cool and moist.
,, 40 ,,	71	64	100·6	99·3	
,, 50 ,,	71	65	100·7	99·3	
,, 60 ,,	71	65	101·2	99·3	
,, 70 ,,	71	65	101·3	99·3	
,, 80 ,,	71	65	101·3	99·3	
,, 90 ,,	71	65	101·3	99·3	No dyspnoea or discomfort. Skin cold over body: warmer over face and hands. Moist all over.

Experiment No. XVIII. 20/7/05.

(Similar to No. XVII. C. G. D. as subject.)

Time	Air temp. °F.		Body temp. °F.		Remarks
	Dry bulb	Wet bulb	Rectum	Mouth	
Just before beginning	71	64·5	99·2	99·2	
After 10 mins'. work	—	—	99·7	99·7	
,, 20 ,,	71	65	100·0	99·8	Slight sweating now. Skin cool.
,, 30 ,,	—	—	100·1	99·9	
,, 40 ,,	71	65	100·2	99·9	
,, 50 ,,	—	—	100·3	99·7	
,, 60 ,,	71	65	100·3	99·8	
,, 70 ,,	71·5	65	100·5	99·8	
,, 80 ,,	—	—	100·6	99·7	
,, 90 ,,	71·5	65	100·6	99·8	No discomfort: slight sweating. Skin cool.

In the two following experiments the work was performed in an air-current produced by an electric fan placed about 10 feet away in the room. The mean air-current at the chest level was measured by holding the anemometer in the hand during ascent and descent of the step-ladder, as during an experiment, and was found to be 135 linear feet (41 metres) per minute. The clothing was as in the previous experiments. In experiment XIX the body temperature was somewhat high at the start, owing to the fact that a previous start had been made about an hour before, and the experiment had been interrupted by stoppage of the fan owing to a defect in a switch.

EXPERIMENT No. XIX. 19/7/05.

(Work in an air-current. J. S. H. as subject.)

Time	Air temp. °F. Wet bulb	Air temp. °F. Dry bulb	Body temp. °F. Rectum	Body temp. °F. Mouth	Remarks
Just before beginning	—	—	100·5	99·0	
After 10 mins'. work	85	97	100·5	99·4	Beginning to drip from sweating.
,, 20 ,,	86	97	100·5	99·6	Sweating profusely.
,, 30 ,,	86·5	97·5	101·2	100·0	
,, 40 ,,	87	97·5	101·2	100·1	
,, 50 ,,	87	97	101·7	100·4	
,, 60 ,,	87·5	97	101·9	100·6	
,, 70 ,,	86·5	97	102·1	100·7	
,, 80 ,,	87	97·5	102·3	101·0	No dyspnoea or distress.
,, 90 ,,	87	97·5	102·5	101·2	Stopped, and remained standing in air-current for 20 mins.
5 mins. after stopping	—	—	102·5	100·5	
10 ,, ,,	86	96	102·5	100·5	Air temp. taken at chest level.
15 ,, ,,	—	—	102·2	100·4	
20 ,, ,,	86	96	102·0	99·4	No feeling of exhaustion after the experiment.

EXPERIMENT No. XX. 19/7/05.

(Same conditions. C. G. D. as subject.)

Time	Air temp. °F. Dry bulb	Air temp. °F. Wet bulb	Body temp. °F. Rectum	Body temp. °F. Mouth	Remarks
Just before starting	—	—	99·8	99·7	
After 10 mins'. work	99	87	100·1	100·2	
,, 20 ,,	99·5	87·5	100·8	100·7	Sweating profusely.
,, 30 ,,	99·5	88	101·1	101·2	
,, 40 ,,	99·5	88	101·6	101·6	
,, 50 ,,	99	88	102·1	102·1	
,, 60 ,,	99	88	102·4	102·3	
5 mins. after stopping	99	88	102·2	101·7	Had stayed in air-current.
25 ,, ,,	71	60	101·8	100·8	Had stayed in cool room a few minutes.

The next two experiments were made with the object of ascertaining the effects of adding a light flannel shirt to the scanty clothing previously worn; and the subjects were clothed in boating flannels (light white flannel shirt and trousers), together with boots and socks. The air was still.

Experiment No. XXI. 22/7/05.

(Work in flannel shirt and trousers. J. S. H. as subject.)

Time	Air temp. °F. Dry bulb	Wet bulb	Body temp. °F. Rectum	Mouth	Remarks
Just before beginning	—	—	99·9	98·5	
After 10 mins'. work	90·5	78·5	99·9	99·1	Dripping from face after 5 mins. Sweating very profusely after 10 minutes.
,, 19 ,,	90·5	79	100·4	99·4	
,, 28 ,,	91	79·5	101·0	100·0	
,, 37 ,,	91	80	101·6	100·3	Pulse 150, standing.
,, 46 ,,	90·5	80	102·0	100·8	
,, 55 ,,	89	80·5	102·4	101·2	Pulse 150, standing.
,, 64 ,,	90	79·5	103·0	101·4	Dyspnoea becoming prominent. Stopped and sat down in room.
3 mins. after stopping	—	—	—	—	Pulse 126, sitting.
5 ,, ,,	—	—	103·2	102·0	Pulse 120, sitting. Respirations 23, and decidedly deep.
12 ,, ,,	90	79·5	103·2	101·8	Pulse 118. Went into cool room.
20 ,, ,,	71·5	64	103·2	100·4	Pulse 106, sitting.
26 ,, ,,	—	—	102·6	99·9	
32 ,, ,,	—	—	102·2	99·7	

Experiment No. XXII. 21/7/05.

(Work in flannel shirt and trousers, cool room. C. G. D. as subject.)

Time	Air temp. °F. Dry bulb	Wet bulb	Body temp. °F. Rectum	Mouth	Remarks
Just before beginning	72·5	64·5	99·7	99·5	
After 10 mins'. work	72·5	64·5	99·5	99·2	Skin damp.
,, 19 ,,	73	65	99·7	99·3	
,, 28 ,,	73	65	100·0	99·4	Sweating pretty freely.
,, 37 ,,	73	65·5	100·3	99·4	
,, 46 ,,	73	65·5	100·4	99·6	
,, 55 ,,	73	66	100·6	99·7	
,, 64 ,,	73	66	100·3	99·8	
,, 73 ,,	73	66	100·4	99·8	
,, 82 ,,	73	66	100·6	99·9	No discomfort.

On comparing experiments XXI and XXII with XIV and XVII it will be seen that the addition of a flannel shirt made little or no difference to the effect of the work on the body temperature.

To further test the influence of flannel clothing the following two experiments were made during rest, the subject being clad in flannel shirt and trousers as before, but with the addition of a flannel boating coat (" blazer ").

Experiment No. XXIII. 20/7/05.

(Rest, sitting, in flannel coat, shirt, and trousers. J. S. H. as subject.)

Time	Air temp. °F.		Body temp. °F.		Remarks
	Dry bulb	Wet bulb	Rectum	Mouth	
Just before beginning	—	—	99·5	98·2	
After 5 minutes	93	80	—	98·3	Face and hands moist.
,, 10 ,,	—	—	99·5	98·4	Whole skin very moist.
,, 20 ,,	93	80	99·5	98·7	
,, 40 ,,	93	80	99·5	98·7	Dripping from face. Wet all over.
,, 60 ,,	93	80·5	99·6	98·8	Pulse 83, sitting. Feel very warm and wet, but no discomfort otherwise.
,, 85 ,,	93	80·5	99·5	98·6	

Experiment No. XXIV. 21/7/05.

(Rest, sitting, in flannel coat, shirt, and trousers. J. S. H. as subject.)

Time	Air temp. °F.		Body temp. °F.		Remarks
	Dry bulb	Wet bulb	Rectum	Mouth	
Just before beginning	—	—	99·8	98·7	
After 5 minutes	94	84	—	98·7	Face very wet, and hands moist.
,, 10 ,,	94·5	84·5	99·8	98·8	Sweat dripping rapidly from face, and whole skin wet.
,, 20 ,,	94·5	84·5	99·8	99·0	Sweating profusely.
,, 30 ,,	94·5	85·5	99·8	99·1	Pulse 85, sitting.
,, 50 ,,	94·5	86	99·8	99·1	
,, 70 ,,	94·5	86	99·8	99·2	Sweating profusely, but no discomfort. Pulse 90, sitting. Clothes damp. Gas turned up.
,, 80 ,,	95	87	—	99·2	
,, 90 ,,	95	87	100·0	99·4	
,, 100 ,,	95·5	87	100·0	99·5	
,, 110 ,,	96	88	100·5	99·6	Pulse 94, sitting.
,, 120 ,,	96	88·5	100·6	99·8	
,, 130 ,,	96	88·5	100·6	99·8	
,, 140 ,,	96	88·5	101·1	100·1	
,, 145 ,,	96	88·5	101·2	100·1	Feel very hot and wet. Not so comfortable.

In a further paper I propose to discuss fully a number of points arising out of the results of these experiments, and to communicate additional experimental data. Some of the broad conclusions may, however, be referred to meanwhile.

In the first place it is evident that the rectal temperature did not show any abnormal increase during rest in still air until the temperature by the wet-bulb thermometer reached about 88° F. (31° C.), provided the subjects were stripped to the waist or clad in light flannel (experiments IV, first part of V and VI, XIII, and first part of XXIV). If, however, the wet-bulb temperature exceeded this temperature by even

1 degree a very marked rise in rectal temperature occurred. This was observed in each of the subjects investigated, and took place whether the air temperature was the same as the wet-bulb temperature (experiment II) or 50° F. (28° C.) above it (experiment XII), or only about 10° F. above it (experiments V and VI, second parts, VII). It was also remarkable that the rectal temperature continued to rise, hour after hour (experiment II), instead of becoming steady after a short time, as might have been expected. In proportion as the wet-bulb temperature rose beyond 88° F. by wet bulb the rise of rectal temperature became more and more rapid. Thus at 89° to 90° F. wet bulb the rise was about 1·0 to 1·4° F. (·5 to 75° C.) per hour (experiments II, VI, VII, XII): at about 94° F. (34·4° C.) the rise was about 2° F. (1·1° C.) per hour (experiments I, VIII): and at 98° F. (36·4° C.) the rise was about 4° F. (2·2° C.) per hour (experiment XI).

In moving air (with the wet bulb still below the body temperature) a higher wet-bulb temperature could be borne without abnormal rise of rectal temperature. Thus in an air-current of about 170 linear feet (51 metres) per minute a wet-bulb temperature up to about 93° F. (34·4° C.) could be borne without abnormal rise of body temperature (experiment IX).

During muscular work in still air the limit of wet-bulb temperature which could be borne without abnormal rise of body temperature was much lower. Thus during leisurely climbing work (13 feet per minute) the limit for a person stripped to the waist was about 78° F. (25·5° C.), or 10° F. lower than during rest (experiments XV, XVI, XVII, XVIII, XXI, XXII); and with harder work this limit would certainly be lower. At a wet-bulb temperature of about 87° (30·5°) the rectal temperature rose about 3·5° F. in an hour (experiment XVI). In an air-current of about 135 linear feet per minute a wet-bulb temperature of about 85° (29·5°) could be borne without abnormal rise of body temperature, but 87° (30·5°) was beyond the limit (experiment XIX).

The symptoms observed to accompany the rise of body temperature were (1) a marked increase in the pulse-rate, accompanied by throbbing in the head; (2) dyspnoea, particularly on any exertion; and (3) a general feeling of exhaustion and discomfort.

Judging from the various observations recorded, the increase in pulse-rate was usually about 20 beats per minute for each 1° F. of increase in rectal temperature, or 36 beats for each 1° C. while the subject was standing in the warm air, the increase being about a fourth less in the sitting posture. On returning to cool air there was, however, an im-

mediate drop in the pulse-rate, so that the increase in the pulse-rate was only about 10 beats per minute per 1° F. of increased rectal temperature in the standing position, and 8 beats sitting. The increase in pulse-rate thus depended not merely on the rectal temperature, but also on the external (wet-bulb) temperature.

The hyperpnoea was not noticeable until the rectal temperature exceeded 102° F. At 103° it was marked during muscular work, and distinctly noticeable during rest. The immediate causes of the hyperpnoea, sweating, &c., will be discussed later.

The discomforts produced by high temperature undoubtedly depend to some extent on other causes than the rise of body temperature as indicated by a rectal thermometer. It is well known, for instance, that persons going for the first time into warm places in mines, or Turkish baths, &c., are very apt to faint or experience nausea or other discomforts. These effects often occur before the rectal temperature can have risen beyond normal limits. Thus in experiment XI, C. G. D. became faint after 20 minutes' exposure to a heat of 165° F. (dry bulb) and 98° F. (wet bulb), though his rectal temperature had only risen to 101·4° F. He had never experienced high temperatures previously; and in subsequent experiments a corresponding rise in his rectal temperature produced no discomfort.

The bearing of these experiments on the question as to the rise in temperature allowable on economic or humanitarian grounds in places where persons have to work continuously will be sufficiently evident. It is clear that in still and warm air what matters to the persons present is neither the temperature of the air, nor its relative saturation, nor the absolute percentage of aqueous vapour present, but the temperature shown by the wet-bulb thermometer. If this exceeds a certain point (about 78° F. or 25·5° C.) continuous hard work becomes impracticable; and beyond about 88° F. or 31° C. it becomes impracticable for ordinary persons even to stay for long periods in such air, although practice may increase to some extent the limit which can be tolerated. In moving air, on the other hand, the limit is extended upwards by several degrees. The men working a rock-drill in a hot "end" or "rise" in a mine, for instance, have the great advantage that the air is kept in constant motion by the exhaust from the drill; and that as this exhaust air is very dry the wet-bulb temperature at the working place is considerably reduced, even if the rock be wet or damped by a jet or spray of water to prevent dust.

STATISTICAL STUDIES IN IMMUNITY. NATURAL IMMUNITY AND THE CAPACITY FOR ACQUIRING IMMUNITY IN THE ACUTE INFECTIOUS DISEASES.

By JOHN BROWNLEE, M.A., M.D. (Glasgow).

The subject-matter of this paper is a study of the relationship which exists between the natural immunity enjoyed by an individual to any disease, and the capacity which he possesses of acquiring immunity if attacked. Most of the facts discussed are moderately well known. They are furnished by the statistics of the acute infectious diseases which have accumulated since the middle of last century. These statistics are in many respects very defective for the purpose required, but they refer to very considerable numbers of cases, and thus afford a basis for the estimation of different degrees of immunity with some accuracy. The most numerous collection of statistics relates to small-pox among the vaccinated and unvaccinated. These have been dealt with in an earlier paper[1], and the results alone will be referred to here. This group of statistics unfortunately practically exhausts the whole data concerning acquired immunity. The next statistics of importance are those referring to the different outbreaks of typhus, relapsing, and enteric fever, and of a few other less common diseases. Last must be placed those regarding the diseases which chiefly affect children. These for several reasons, however, afford less definite data.

The causation of an epidemic must depend on the capacity which an organism possesses for infecting, as much as on the susceptibility of the population attacked. The theory that an epidemic comes to an end because all the susceptible persons have been attacked has been rejected by most epidemiologists. The cause of the decline must rather be sought for in the loss of virulence of the organism or of its infecting power.

[1] *Biometrika*, Vol. IV.

The term "virulence of an organism," however, is one which is not clearly defined. It seems to be applied to the condition in which a considerable degree of infecting power is associated with a capacity for producing a severe type of disease. A marked capacity for producing a severe attack of a disease may, however, be associated with a very low degree of infectivity, while the presence of great power of infectivity in an organism does not imply that the type of disease produced will be necessarily severe. Examples of these conditions occur at once to the mind of all epidemiologists, *e.g.* the recent extensive mild epidemics of small-pox in America while in Britain and France the severity was very considerable.

The statistics which are available for the study of this point are not very numerous. Deaths have been accurately recorded for a large number of years, but compulsory notification of disease has been in force only for comparatively few, so that except in a few instances the fatality of any disease can only be ascertained for short periods. The yearly numbers of cases and deaths afford valuable information. They show the long waves of disease, and though the deaths may not be quite accurately assigned to the actual cases, yet, as the duration of infectious disease in fatal cases is as a rule short in comparison with the length of the year, no error of any moment is introduced. The comparison of the amount of any infectious disease and its fatality during the same series of years also yields some information; and lastly hospital statistics which exhibit the monthly numbers of cases, with the deaths which occur among these cases truly assigned to them, afford a means of tracing the severity of epidemics from start to finish. Unfortunately such are somewhat scanty. Most returns state such facts as the weekly number of cases and the weekly number of deaths, though the latter refer largely to different cases. The effect of this is to lessen the fatality during the rise of an epidemic and to exaggerate it during the decline. Even when, as is sometimes done with scarlet fever, the deaths of the week later are taken for comparison we get only a rough approximation to the truth.

The figures contained in the illustrative tables are taken chiefly from Dr Newsholme's work on "Epidemic Diphtheria" (with additions kindly supplied by himself), from the Report of the Medical Officer of the Local Government Board, 1899–1900 (pp. 268 *et seq.*), and from the Reports of the City of Glasgow Fever Hospitals 1865–1904. The Report of the Medical Officer of the Local Government Board referred to contains tables showing the number of cases notified and the number of deaths

from scarlet fever, enteric fever, and diphtheria, in seventy-six English towns for the five years 1893–1897.

TABLE A.

Number of years in which diphtheria has been epidemic classified according to prevalence of the disease and the fatality.

(*a*) NORWAY, 1867–1891.

Fatality %	Case rates per 100,000 0—100	100—200	200—300	300—400	400—500	500—600
13—18	4	2	—	—	—	—
18—21	3	—	—	—	—	—
21—24	1	3*[1]	—	4	—	1
24—27	1	1	1	2	2	—

(*b*) COPENHAGEN, 1855–1894.

Fatality %	Case rates per 100,000. 0—200	200—400	400—600	600—
10—12	—	5	—	6
12—14	2	1	2	1
14—16	1	3	1	2
16—20	—	4	1	1
20—	9	1	—	—

As the natural histories of infectious diseases present different characteristics it will be better in arranging the evidence to discuss each disease separately. With regard in the first place to diphtheria, it is to be noted that in the towns of England where the disease is least prevalent it is most fatal. This might well be a fictitious result, depending on the likelihood that where the disease is most common the serum treatment will be more efficaciously carried out, while mild cases will be more certainly recognised. A like relation, however, is observed to hold when the numbers of cases and deaths from diphtheria occurring in Copenhagen (Table A) for the series of years 1855–1894 are examined. On the other hand, the statistics of Norway and of Hamburg show the opposite tendency, the epidemic years being associated with a definitely higher case mortality. The factors of infectivity and virulence are thus capable of existing in very different degrees of association. I am not able to trace any individual outbreak of diphtheria from beginning to end and consequently cannot make any observations as to how the fatality varies as a single epidemic proceeds from start to finish.

[1] In explanation of table for instance the 3 marked by an asterisk indicates that in three years the amount of diphtheria was between 100 and 200 per 100,000 of population, and the fatality, *i.e.* the case mortality, between 21 and 24 %.

Table B.[1]

Table showing how the fatalities of certain diseases vary during the course of epidemics.

Enteric Fever—Glasgow, 1898–1899.

Months	Cases	Deaths	Fatality %
June, July	68	17	25·0
Aug., Sept.	233	44	18·8
Oct., Nov.	338	56	16·5
Dec., Jan.	145	29	20·0
Feb., Mar.	115	15	13·1
April, May	103	13	12·6

Enteric Fever—Glasgow, 1899–1900.

Months	Cases	Deaths	Fatality %
June, July	98	17	17·4
Aug., Sept.	275	42	15·3
Oct., Nov.	206	40	19·4
Dec., Jan.	106	21	19·8
Feb., Mar.	93	18	19·4
April, May	49	11	22·5

Scarlet Fever—Glasgow, 1886–1895.

Months	Cases	Deaths	Fatality %
Jan., Feb.	2,063	147	7·1
Mar., April	1,463	106	7·4
May, June	1,591	127	8·3
July, Aug.	2,079	149	7·2
Sept., Oct.	3,820	231	6·1[2]
Nov., Dec.	2,994	218	7·3

Measles—Glasgow, 1896–1899.

Period of Epidemics		Cases	Deaths	Fatality %	
Months before	5	43	3	7·0	5·8
	4	99	8	8·1	
	3	162	10	6·2	
	2	315	15	4·8	
	1	522	59	11·3	10·5
Centre of Epidemic		743	71	9·6	
Months after	1	625	63	10·1	
	2	[illegible]	57	11·9	
	3	338	23	6·8	6·6
	4	229	11	4·8	
	5	83	11	13·2	
	6	64	3	4·7	

[1] These tables have not been corrected for age differences. In the case of scarlet fever and small-pox in Glasgow the correction was found to be negligible.

[2] The same low fatality in September and October is seen also in the statistics of London, *vide* Reports by Sir Shirley Murphy.

Relapsing Fever—Glasgow, 1870-2.

Months	Cases	Deaths	Fatality %
June—Aug.	161	4	2·5
Sept.—Nov.	822	11	1·4
Dec.—Feb.	1,470	34	2·3
Mar.—May	739	16	2·3
June—Aug.	496	7	1·4
Sept.—Nov.	700	16	2·3
Dec.—Feb.	313	9	2·1

Small-pox—Gloucester, 1896.

Weeks ending	Vaccinated			Unvaccinated		
	Cases	Deaths	Fatality %	Cases	Deaths	Fatality %
Feb. 1—22	43	6	13·9 } 8·8	75	34	45·3
,, 29—21 Mar.	243	19	7·8 }	173	78	45·6
Mar. 28—18 Apr.	440	43	9·8	221	91	41·2
Apr. 25—16 May	240	24 } 8·1		117	49	42·0
May 25—13 Jan.	106	4 }		50	22 } 40·0	
Jan. 20—11 July	32	1 } 8·0		11	2 }	
July 11—	5	2 }				

Small-pox—Glasgow, 1900-1901.

Periods			Vaccinated.		
			Cases	Deaths	Fatality %
First outburst,		Apr.—Aug. 1900	195	6	3·06
Second	,,	Jan. 1901	405	28	6·9
Third	,,	Feb. 20—28	133	7	5·2
,,	,,	Mar. 1—31	394	37	9·3
,,	,,	Apr. 1—20	99	18	18·3

In enteric fever (Table B) a somewhat similar relationship between size of epidemic and severity of attack holds with regard to the large towns of England, and when the statistics of Glasgow for the last forty years are considered it is found that in general the years of epidemic have been the years of lowest case mortality or fatality. When, however, a like comparison is made between the prevalence of the disease and its fatality in London exactly the opposite relationship is observed. The experience of one town for a series of years is specially valuable as the personal equation of different observers is to some extent eliminated, a consideration which makes this divergence more interesting. The course of severity of single epidemics of enteric fever can be easily traced for Glasgow. When the cases occurring in the epidemics during a series of years are gathered together according to the corresponding dates it is found in the sum that the period of greatest prevalence is the period of lowest mortality. This is not necessarily the case in any individual

epidemic. In the two tabulated a minimum mortality occurs during the maximum period of prevalence, but in the first of these, the beginning, and in the second, the end was the period during which the case mortality was highest.

The behaviour of scarlet fever exhibits some points of similarity with that of enteric fever. Here again the period of maximum prevalence in the autumn is associated with a lower case mortality than that in the rest of the year. There is, however, very little indication that in the epidemic periods it is more or less severe than in the inter-epidemic periods to any marked degree. For a series of years in any one place the data are hardly comparable, as the disease has shown such marked variations in fatality in recent years, but a comparison of the mortality in the towns of England 1892–97 indicates that the disease was slightly more severe in those towns in which it was most prevalent.

Regarding measles it is more difficult to obtain satisfactory information. If epidemics do not come at regular intervals then the age incidence of the cases varies so much from epidemic to epidemic that it is impossible to compare their crude death-rates, even if the number of cases were accurately known. The course of individual epidemics has seldom been traced, and the only figures which allow of tabulation are those of the Glasgow Fever Hospitals. A consideration of the figures in which five of the last severe epidemics of measles are gathered together indicates that the middle of the epidemic has been the time of greatest fatality.

As a last example, instances of small-pox may be cited. In the Gloucester epidemic in 1895 when the vaccinated and unvaccinated cases are grouped together the onset of the epidemic was apparently more severe than its decline, yet when the two are considered separately it is seen that throughout the fatality among the unvaccinated was approximately constant, while among the vaccinated there was no great variation. The explanation of the seeming initial severity is thus to be found in the fact that the unvaccinated were comparatively much more numerous in the earlier than in the latter ages of the epidemic. In Glasgow on the other hand, in 1900–1901, the epidemic became progressively more severe from the start to finish, and towards its close was nearly six times more fatal than at its commencement.

Another instance referring to relapsing fever is given in the table but does not call for any special comment. There is no indication, for instance, that during the epidemics of relapsing fever in Glasgow, 1870–72, any period of the epidemic was characterised by any special

fatality. On the other hand, in the extensive outbreaks of typhus fever in Ireland, 1817–19, the year 1818, which represented the height of the epidemic, was marked by a lower fatality than that of the years immediately preceding and following. These instances show that in general there is no constant relationship between the infective power of an organism and its capacity for producing severe disease, and that in the same epidemics, at the same places, an organism may be most lethal during any period in the epidemic. In particular it is to be noted that during the latter periods of an epidemic when it might be surmised *à priori* that the severity would decrease the reverse is frequently found to be the case.

This leads to the consideration of the subject from the point of view of the attacked organism. Immunity is commonly considered as being of two kinds, natural and acquired. While this is quite true and while both of these forms of immunity have long been known to exist it would seem that the division should not be made between natural and acquired immunity, but between natural immunity and the capacity for acquiring immunity. These constitute two quite distinct properties, and are the properties in the invaded organism corresponding to those in the attacking organism just differentiated as infectivity and virulence. For the first is evidently the power which the invaded organism possesses of protecting itself against attack, while the latter is the power, if entry be established, of overcoming the virulence of the attacking organism and re-establishing health. Whether the acquired immunity which this implies is purely temporary or permanent is of no moment as regards the presence or absence of the capacity referred to.

These two properties, like infectivity and virulence, may exist in very varying degrees; natural immunity may be very high and the capacity for acquiring immunity very low, *e.g.*, glanders in man; or the reverse may be the case, the susceptibility being extreme, and yet the fatality almost non-existent, *e.g.*, relapsing fever or chicken-pox. Both may be present in a very slight degree (given the natural conditions of infection) as in plague, or cholera. The fourth possibility, high natural immunity combined with high capacity of acquiring immunity, is not so easily illustrated. Such diseases must necessarily be of rare occurrence and low mortality, and may consequently have largely escaped notice. Pfeiffer's glandular fever of children possibly approximates most nearly to this combination.

Before passing to the description of the manner in which these different properties vary as regards their presence or absence it is

necessary to define the numbers on which the comparison is based. The ratio between the number of cases and the population may be considered as the measure of the prevalence of or susceptibility to any infectious disease; for purposes of convenience this number may be multiplied by some power of ten. The natural immunity obviously bears some inverse relationship to this number, while from the point of view of the attacking organism the infectivity has evidently some direct relationship.

The ratio between the number of deaths from any disease and the number of cases among which these deaths have occurred, may likewise be considered as the measure of the severity of the disease. This number bears a direct relationship to the virulence of the organism and an inverse relationship to the capacity for acquiring immunity. It is commonly known as the death-rate from the disease, or more correctly as the case mortality, but to avoid confusion it will be referred to under the term of the "fatality" throughout this paper.

A third ratio requires also to be noted. If the number of deaths be divided by the population we obtain a number which in this paper will be referred to as the "mortality" from the disease. This may also for purposes of convenience be multiplied by some power of ten. These ratios are not independent, and if any two be known the third can immediately be calculated. For the purposes required in this paper the first two are most important, but the third as throwing light on some of the problems will be frequently referred to.

In all that is said hereafter it must be clearly understood that averages alone are dealt with. There is no clinical observer of fevers with any experience but will be able to produce any number of examples of patients who differ to a very considerable extent in the relative and absolute quantities in which they possess the different properties of immunity here discussed from the average presented by all persons of the same age. But it must be remembered that this method of bringing individual exceptions of more or less diversity from the type is no argument against the type representing a fact, divergences on either side of the average being the rule in all biological measurements.

The statistics of the continued fevers, typhus, typhoid, and relapsing fever afford good examples of the relationship which exists between the three ratios just discussed, and in association with these miliary fever or sweating sickness may also be considered. The accompanying table (Table C) gives the series of figures which are obtained when the susceptibility, fatality, and mortality ratios are calculated for each age

period for epidemics of these diseases. These figures with slight and unimportant differences hold true generally for all the epidemics examined.

Table C.

Table showing susceptibility, mortality, and fatality of typical epidemics of various diseases.

Typhus Fever—Glasgow, 1865–1872.

Age periods	Susceptibility	Mortality	Fatality %
0—1, 1—5	422	32	7·6
5—10	1578	21	1·3
10—15	2440	44	1·8
15—20	2060	140	6·8
20—25	1330	143	10·8
25—30, 30—35	1025	179	17·5
35—40, 40—45	1167	319	28·2
45—50, 50—55	804	343	41·1
55—60, 60—65	466	248	53·2
65—70, 70—75	120	154	80·0
75—80, 80—	—	—	—

Relapsing Fever—Glasgow, 1870–72.

0—1, 1—5	374	169	4·5
5—10	1066	38	·4
10—15	1495	21	·1
15—20	1601	62	·4
20—25	1153	79	·7
25—30, 30—35	1916	207	2·3
35—40, 40—45	1131	322	2·8
45—50, 50—55	955	549	5·7
55—60, 60—65	605	741	12·2
65—70, 70—75	259	579	22·2
75—80, 80—	—	—	—

Enteric Fever—Glasgow, 1895-1903.

Age periods	Susceptibility	Mortality	Fatality %
0—1, 1—5	309	22	7·1
5—10	1216	94	7·7
10—15	1321	139	10·6
15—20	991	182	18·4
20—25	1043	229	22·5
25—30, 30—35	830	221	26·6
35—40, 40—45	437	137	31·3
45—50, 50—55	148	41	27·8
55—60, 60—65	90	30	33·3
65—70, 70—75	13	13	100·0
75—80, 80—	—	—	—

Miliary Fever[1]—Oise, 1821.

0—1, 1—5, 5—10	113	88	7·8
10—15, 15—20	393	56	1·46
20—25, 25—30	1019	621	6·0
30—35, 35—40	1283	742	5·8
40—45, 45—50	1199	497	4·2
50—55, 55—60	989	566	5·7
60—65, 65—70	739	439	5·9
70—75, 75—80, 80—	242	180	6·7

[1] Rayer, *Suette Miliare en* 1821. Paris, 1822.

Small-pox Unvaccinated[1]—Sheffield, 1887–1888.

Age periods	Susceptibility	Mortality	Fatality %
0—1	54	27	50·5
1—5	332	117	33·6
5—10	577	122	21·2
10—15	682	133	19·6
15—20	626	173	28·6
20—25	271	109	39·5
25—30	73	24	33·3
30—35	90	38	42·4
35—40	59	26	43·5
40—45	24	9	37·5
45—50	26	15	57·1
50—55	7	3·5	50·1
55—60	4	—	—
60—65	6	4	66·6
65—70	—	—	—
70—75	—	—	—
75—80	—	—	—
80—	—	—	—

For typhus and relapsing fever the relationship shown is almost identical. (See diagram 1 and table C.) The susceptibility is greatest at the same ages, namely, ten to twenty years, and from this period

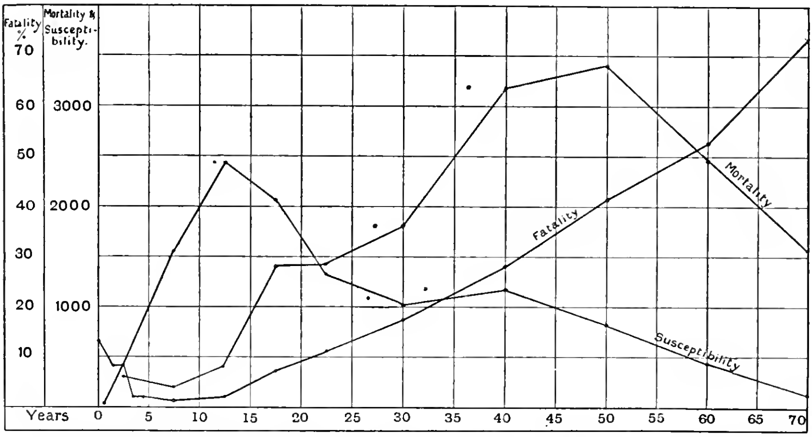

Diagram I. Showing the course of the Susceptibility, Mortality, and Fatality Curves for Typhus Fever. (Glasgow, 1865–72.)

[1] These figures are based on the actual number of persons found to be unvaccinated when the census of the state of the population as regards vaccination was taken. (See Local Government Board Report by Dr Barry.)

towards youth and old age there is a gradual fall. The fatality bears an inverse relationship to this, being comparatively high among persons under five years, specially low during the period of maximum prevalence, and thence progressively increasing as the age becomes greater. The mortality in both shows a similar course, being high during the first five years, thence decreasing to a minimum about the period of greatest prevalence. It thereafter increases till it reaches a maximum during the ages of manhood or commencing old age and then declines. Enteric fever shows some differences from this. Its prevalence is much the same. The fatality, however, shows no special tendency to be high during the early years of life, and increases more or less steadily from youth to old age. The mortality curve of enteric fever has its maximum earliest of the continued fevers, this occurring between the ages of fifteen and twenty-five years. Miliary fever has its period of greatest prevalence somewhat later, namely, between thirty and forty years, and the mortality also reaches its maximum at this time. The fatality varies remarkably little, and, with the exception of the years five to twenty, showing a minimum almost discontinuous with the years before and after its constancy, is a most striking feature.

These figures have all been calculated on the actual populations living at each age at the nearest census, and are open to the objection that the lessened prevalence at higher ages not being calculated on the number of those who are actually susceptible becomes progressively more inaccurate from youth to old age, but the numbers of those who have passed through an attack of any of those diseases is always so small compared with the total population, even at high ages, that its effect may be neglected as producing a small absolute error in nowise affecting the general truth of the figures. With regard to relapsing fever the objection is of no moment, as the disease seems to confer practically no permanent immunity, so that a population fifteen years after one epidemic is practically virgin soil, while like remarks are also true with reference to miliary fever.

In the diseases of children there is some difficulty in obtaining accurate information regarding the period of maximum susceptibility. Two of them, measles and whooping-cough, are so common in early years and affect such a large percentage of the total children in the population, that by the time ten years of age is past there can be very few susceptible persons left. Thus, out of 1,245 consecutive cases of measles admitted to the hospital whose birth-place was ascertained, only 88 were above the age of ten years, and of these 88 only ten were

born in Glasgow, the rest coming from country districts where measles is only present at intervals, so that the age of maximum susceptibility as shown in the statistics may probably differ largely from that which would be the case if the infection fell upon a population uniformly susceptible at all ages. The same remarks apply to whooping-cough and small-pox, and will be more fully illustrated with regard to the latter.

Scarlet fever and diphtheria, however, are so much less prevalent as to permit of the relationship of the constants being easily displayed with comparative accuracy. The average number of cases of scarlet fever in Glasgow is about 3,500 a year; of these only a tenth occur after the age of twenty years and an average of 6 % die, so that for the five yearly age period twenty to twenty-five years, there will only be living as an absolute maximum 15,000 persons who have passed through scarlet fever. This is calculated on the assumption that none have died up to this age of any other disease since their recovery from scarlet fever. Now as the population of Glasgow at this age period in 1901 was 83,319 it is evident that any error in the prevalence or mortality ratios will not exceed a fifth, nor unless the fact of having passed through scarlet fever either lengthens or diminishes subsequent chance of life will the error tend to vary much at higher ages from this amount. The census population thus affords a sufficiently accurate means of determining the prevalence and mortality ratios. The fatality ratio is quite independent of the population.

The accompanying table (Table D) gives these ratios for diphtheria and scarlet fever taken from the returns of Manchester. These are chosen as they are the only returns where the actually notified cases and the corresponding deaths are distributed in age periods. It is evident that a very marked difference from the relationships which have hitherto been observed exists here. The maximum period of susceptibility falls in youth and thereafter steadily declines, while a like relationship holds with regard to the mortality. In both these diseases the maximum of the latter occurs before the maximum of the former, thus showing a difference to what has been observed in the statistics of the continued fevers considered above. The fatality in both is specially high in infancy, and then steadily declines, and there is some indication that this reaches its minimum at the age of puberty, and that from thence to old age increases, though not to the same extent as that shown in the continued fevers. A like minimum is shown in the statistics of London and Glasgow. Children's infectious

TABLE D.

Table showing the Susceptibility, Mortality, and Fatality of Scarlet Fever and Diphtheria. Manchester, 1893—1903.

Scarlet Fever.				Diphtheria.			
Age Period	Suscepti-bility	Mortality	Fatality	Age Period	Suscepti-bility	Mortality	Fatality
0—1	205	45	21·5	0—1	89	62	71·2
1—2	671	114	17·1	1—2	271	160	58·4
2—3	1293	186	14·2	2—3	293	152	51·2
3—4	1708	195	11·6	3—4	392	161	40·9
4—5	1924	173	9·0	4—5	356	129	36·1
5—6	1971	93	4·7	5—6	325	100	33·2
6—7	1825	68	3·4	6—7	199	56	27·9
7—8	1675	53	3·1	7—8	187	44	24·6
8—9	1352	25	1·8	8—9	152	34	20·2
9—10	1172	21	1·8	9—10	124	14	11·6
10—15	682	11	1·6	10—15	74	5	7·2
15—20	214	6	2·7	15—20	46	2·1	4·4
20—25	93	3	2·3	20—25	37	1·1	2·8
25—35	44	1	2·3	25—35	29	2·5	4·4
35—45	15	·4	2·9	35—45	16	·6	3·7
45—55	7	·2	3·0	45—55	9	·4	4·8
55—65	3	—	—	55—65	5	—	—

diseases thus present points of very considerable difference to those observed in the diseases which chiefly affect adults[1].

The last disease of importance is small-pox. The facts with regard to this have been examined in a previous paper by myself published in *Biometrika* and need only be given here in *résumé*. Before the introduction of vaccination this was a disease of which the age prevalence and fatality did not differ greatly from those of measles at the present time. Youth seemed to be the period of much the greatest susceptibility, and the disease in any case was so infectious that when it broke out among a population from which it had been absent for a considerable time it attacked such a large proportion of the total persons not protected by previous attack as to render the detection of the period of greatest

[1] These remarks concerning the comparative immunity to scarlet fever possessed by persons over ten years of age apply generally to the manner in which that disease appears in the cities of this country, yet when an epidemic attacks a virgin community the period of high susceptibility does not cease till the age of twenty is reached. This is shown in the epidemic of scarlatina which broke out in the Faroe Islands in 1875, and it raises the question as to whether some degree of immunity may not be obtained by living in a centre where the infection is constantly present, even although an actual attack of the disease is not experienced; this however cannot be dealt with here.

susceptibility somewhat difficult. It had been calculated by Duvillard even at this time that during the years ten to fifteen there must be a slightly higher susceptibility, but the statistics of the recent epidemics, especially that of Sheffield in 1887, of which a census of the population was taken, show that this period is one which exhibits considerably greater susceptibility than Duvillard supposed. The prevalence, mortality, and fatality curves resemble those of relapsing fever and typhus, with the difference that both the susceptibility and the mortality are higher at early years. The chief interest of small-pox statistics, however, lies in their affording a certain amount of evidence on the subject of acquired immunity, which will be considered later.

This completes the tables showing the statistical facts relating to natural immunity, and the capacity for acquiring immunity. So far the diseases belong to two groups, the one including those diseases which chiefly affect adults, and the other those which chiefly affect children. In all the former there is a general agreement. The susceptibility increases from birth up till ten to fifteen years, and thereafter diminishes, while the fatality shows an exactly opposite course. The latter is high in the early years of childhood, decreases till the age period ten to fifteen, and thereafter increases steadily towards age. With enteric fever there is an absence of this special fatality in youth, but with this exception the general course is the same. It would seem as if there was an inverse relationship between the natural immunity and the capacity for acquiring immunity. As the natural immunity increases with age the possession of high capacity for acquiring immunity becomes less necessary. It is to be noted that in the only disease in this group in which the fatality remains practically constant, namely, miliary fever, there is by no means the same increase of natural immunity towards old age, so that protection here also is given with a like economy. That the high death-rate is not due merely to the aging of the tissues, nor to the fact that the respiratory system becomes more liable to break down as age advances, is shown from the instance of miliary fever. Measles, however, affords a better example. Age alone apart from acquired immunity does not bring any special increase of natural immunity, and yet the fatality of measles at high ages remains very low. Out of 12,000 cases of measles treated in the City of Glasgow Fever Hospital, Belvidere, 1885–1902, 71 were over the age of thirty, and of these three died, though the general mortality of all ages was 9 %. These 71 were almost without exception persons born in the country who had not passed through measles in early youth.

This inverse relationship between natural immunity and the capacity for acquiring immunity, so distinctly shown in the diseases which chiefly affect adults, is not nearly so marked a characteristic of the diseases which affect children. As already stated measles and whooping-cough do not lend themselves to this statistical study, so that the figures concerning scarlet fever and diphtheria must be taken to illustrate this type. The facts have been already stated. There is one point of similarity with the continued fevers. In both scarlet fever and diphtheria at ages over fifteen years the inverse relationship under discussion obtains. The natural immunity in both diseases increases from this period, and the fatality also increases, though the increase is very small in proportion to that observed in the continued fevers. It is also much more definite in scarlet fever than in diphtheria. The differences, however, predominate. The maximum susceptibility to scarlet fever is between the years of four and five, while the maximum mortality is between the years of three and four. It is, however, to be noted that from 0–5 years, during which the susceptibility steadily increases, the fatality as steadily declines, and so far the inverse relationship is observed to exist. From the age of five years, however, both fatality and susceptibility diminish together, and it is not until the age of fifteen is reached that they again hold an opposite course. The case of diphtheria presents no essential difference to this. There is thus no absolutely constant relationship between the degrees in which natural immunity and the capacity for acquiring immunity may be present, but they are generally found present in inverse amounts during the different periods of life at which diseases may be acquired. The chief exception to this occurs in the disease of childhood between the age of five and fifteen years. The measure of this inverse relationship is not absolute for disease in general, but requires to be investigated specially for each disease in particular.

Further, some diseases not definitely proved parasitic, such as cancer, display a somewhat similar condition of affairs. It is well known clinically that when this disease developes in a person under thirty years of age it advances with startling rapidity, the organism apparently being unable to bring into play any forces in the least adequate to combat the illness, while if the disease develope later at those periods of life when apparently the susceptibility is greatest a long and, it would seem occasionally, a successful fight may be maintained against the growth of the tumour. Somewhat similar remarks might be made regarding diabetes, but to survey this field would demand the descrip-

tion of much detail on which evidence can be displayed only with doubt and difficulty.

As a general rule it would seem that the two qualities discussed are rarely present at their maximum at the same ages. Nature is a well-known economist. No more material is used for any definite purpose than is required for its adequate fulfilment, and as life in bulk may be readily protected if either natural immunity or the capacity for acquiring immunity is present in a high degree the means are sufficient to the end.

Several explanations may be proposed to explain this association of high natural immunity with low capacity for acquiring immunity in certain instances suggested by cases of the specific fevers. One is that in age the tissues are less resistant to disease, but this has already been rejected for these diseases, and the examples of cancer and diabetes just cited fall in the same way. A second is that a specially virulent bacillus must be present to ensure the infection of a person of high natural immunity. To this the course of the mortality curve offers the best answer (see diagram I), although as has been shown the infectivity and virulence of an organism are not necessarily associated. Were a bacillus of any definite virulence required to produce a severe attack, then the mortality curve would more or less take the form of a straight line, unless some particular age or ages were more exposed to the risk of infection than others, and of this there is no satisfactory evidence in the continued fevers. In the accompanying table (Table E) the mortalities for several zymotic diseases are tabulated as given by the Registrar-General for the decade 1881–91. It is seen that very large variation is exhibited, especially in these diseases which attack children. Though the variation is not so marked with enteric fever and typhus fever as with scarlet fever and diphtheria it is still considerable. The variation, however, of the mortality in the former diseases given by the statistics of fever hospitals where the diagnosis is verified is much more considerable and leads to the conclusion that many deaths occurring in youth are wrongly ascribed to these diseases. When reference is made further back to the decade 1871–81 the age distribution of deaths given by the Registrar-General is totally different for both these fevers from that just cited. There the mortality among children is yet more considerable, and the comparison makes belief in the official figures almost impossible. The variation, considerable in the statistics of the Registrar-General, is thus yet more considerable where opportunity for checking the diagnosis is available, and is too large to admit of the

TABLE E.

Table showing Death-rates (mortality) per 100,000 *for certain fevers. England,* 1881—1891.

Age period	Scarlet Fever	Diphtheria	[1] Enteric Fever	[1] Typhus Fever
0—1	672	282	49	1
1—2	1885	686	108	2
2—3	2115	773	141	3
3—4	2042	896	170	5
4—5	1708	849	187	8
5—10	762	424	180	7
10—15	153	100	208	9
15—20	41	36	289	16
20—25	26	20	282	15
25—35	22	17	232	18
35—45	12	16	182	26
45—55	4	14	155	21
55—65	2	17	142	17
65—75	3	17	113	12
75—	1	12	62	6

explanation holding true. There is besides no disease in which the mortality is constant at all ages.

Exactly the same remarks apply to infection by a large dose of the poison and by the same reasoning it will be seen that persons more or less immune to a poison who become infected are not, taken all over, those who receive a larger dose than the average.

Explanations associated with method of infection do not seem much more satisfactory. If it could be proved that enteric fever in young adults was more commonly acquired by eating shell-fish, for instance, a cause which is said to produce mild attacks, then a certain amount of explanation would be afforded, but such outbreaks seem more commonly to affect adults. With regard to the children's diseases this explanation also fails. Here it might be assumed that the mortality was higher at early ages because children are brought into specially close contact with one another in schools, but the maximum mortality occurs in none of these diseases during school ages, and the variation in the amount of the mortality at different ages is so large as to make such an explanation impossible.

The question of acquired immunity remains to be considered. The only statistics which are at all suitable for the investigation of this are those relating to small-pox and vaccination. Small-pox is a disease

[1] In both of these diseases the Registrar-General's returns give a number of deaths at early ages in considerable excess of what is found in hospital statistics.

which displays some peculiarities; there is the stage of initial fever, associated with very severe constitutional symptoms, which occurs prior to the appearance of the eruption, and which suddenly abates as the rash becomes visible. The secondary fever differs absolutely in its character from the primary. It begins generally when the eruption reaches the stage of vesiculation, increases as the rash developes, and subsides as it dries up. It is this second stage which vaccination clinically resembles and against which the latter affords the greatest protection. In cases of small-pox modified by vaccination it is no uncommon thing to find a primary fever of great severity associated with an almost complete absence of eruptive elements, showing that the toxines produced in the two stages of the disease are in some way considerably different. Being thus a complex disease the statistics of the type of small-pox which follows vaccination allow the decline of acquired immunity to be measured at two levels, (1) strength of protection against attack, and (2) strength of protection against death. It is found that the duration of the protection against attack is very much less than that against death.

The course of both is exhibited in the accompanying diagram as determined from the statistics of the great epidemic of small-pox at Sheffield in the year 1887–88. These figures are calculated from the numbers at each separate age period of the vaccinated and the unvaccinated. On account of the alteration which age produces as regards natural immunity and fatality quite apart from the presence or absence of vaccination it is easily seen that a comparison can only be valid if made in this way. The diagram shows that against attack the protection remains fairly complete until the age of fifteen years, thence it declines rapidly until it is practically absent at the age of thirty, though it never absolutely disappears.

With regard, however, to the protection against death, that is to say, against that stage of the fever to which vaccination clinically corresponds, the decline, though marked from fifteen to thirty years, falls but little after this date and always remains considerable. This latter fact is of specially great importance as indicating that immunity which is acquired and not natural is not only never lost, but though the absolute protection may diminish with old age, yet a great relative protection remains in spite of this.

If the relative natural immunity at different ages among the unvaccinated be measured it is found to vary in the manner shown in diagram II, which, taking the susceptibility of small-pox under one year of age as the standard for comparison, shows in another form what has already been stated when the prevalence of the disease among the

unvaccinated was mentioned earlier in this paper. It is seen from this that the natural immunity is least between the ages of 10 and 15 years. Between the ages of fifteen to twenty years, it is again approximately equal to that during the first year of life and thereafter steadily increases.

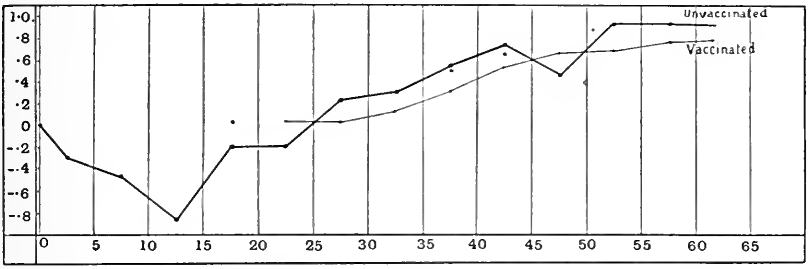

Diagram II. Curves indicating the growth of natural immunities among vaccinated and unvaccinated for the Sheffield Epidemic of Small-pox, 1887–8. The increase of susceptibility is to be noted among the latter up to the age period 10—15 years, and the gradual growth of immunity from that date onwards. Taking the age period 15—20 years as a standard for the vaccinated the growth of natural immunity thence onwards is shown. Remembering that the latter curve starts from a level of acquired immunity still considerable, it is curious to note how similarly natural immunity increases in the vaccinated and unvaccinated alike.

If for the vaccinated the curve representing the combination of natural and acquired immunity is drawn it is seen that the protection against attack is at a minimum between the ages of fifteen and twenty years, and thereafter gradually increases. The minimum between fifteen and twenty years does not represent a period at which the acquired immunity is absent, but taking it as a standard and representing the susceptibility at the succeeding ages in the same way as has been done for the unvaccinated, it will be noted that the increase of natural immunity due to age among the vaccinated as indicated in the diagram, follows very closely that shown by the like curve referring to the unvaccinated, in other words, the development of natural immunity due to age is quite independent of the presence or absence of acquired immunity. Lastly, in this connection it is to be noted that the fatality of the disease increasing with age among persons who have been vaccinated does not indicate a corresponding loss of the protection produced by acquired immunity, because it increases *pari passu* with that among the unvaccinated. This is shown by diagram III, where it is seen that after thirty years of age there is no further relative loss in the protection against death displayed by the vaccinated as against the unvaccinated,

the increase in fatality is due to the lessened capacity for acquiring immunity, which is a feature shown by the human system in regard to small-pox as well as some other diseases, though as before remarked not a necessary consequence of age itself.

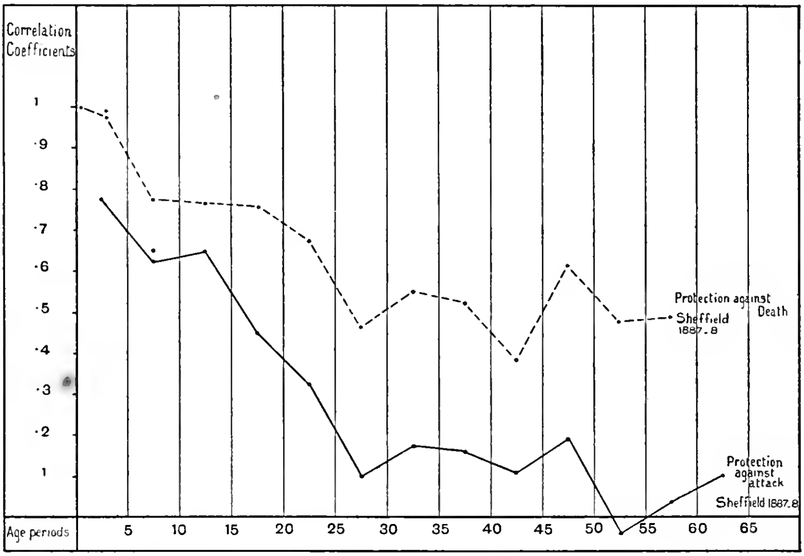

DIAGRAM III. Diagram showing the manner in which the protection afforded by vaccination decreases. The lowest line refers to the protection against attack which it is seen nearly vanishes between the ages of 25 and 30. The upper represents the loss of protection against death. It is to be noted that while this tends to fall rapidly up to the age period 25—30 years, thereafter there is no further loss, so that some permanent change is made in the tissues by vaccination. The statistics for other towns show essentially the same phenomena.

In conclusion, to bring the argument in the preceding pages into line with the experimental theory of immunity the following points may be noted. There are at present two different means known by which an organism is protected against infective agents, one independent of the character of the body fluids, and the other dependent on the actual presence or rapid production of certain protective agents in these fluids. The former is seen, for example, in the immunity possessed by fowls to the toxin of tetanus which is not the result of any known chemical reaction of the tissues or fluids of the body. Of the second the immunity of the horse towards diphtheria is an example; for though it may be said that the horse is an animal naturally immune to this disease, it would appear that it is rather an animal which possesses a remarkably small amount

of natural immunity in the sense in which this is exhibited by the fowl against the poison of tetanus, but on the other hand possesses a capacity for acquiring immunity of such amount as to render its defence against the disease practically absolute. It would probably be better to restrict the term natural immunity to the former of these and to use some other term to describe the latter. To correlate these two properties with the two protective mechanisms which must be inferred to exist from a consideration of the statistics of the infectious diseases, is, of course, conjectural, but it seems more rational to infer that they are manifestations of the processes known experimentally to exist rather than to suggest other explanations.

RELATING TO THE PAPER ENTITLED "THE SUCCESSFUL APPLICATION OF PREVENTIVE MEASURES AGAINST BERI-BERI," BY DR HAMILTON WRIGHT.

LETTER RECEIVED FROM

DR G. A. O. TRAVERS.

KUALA LUMPUR,
SELANGOR,
MALAY PENINSULA,
18*th August*, 1905.

DEAR SIR,

I have read with much interest an article on Beri-beri by Dr Hamilton Wright, M.D., C.M. (McGill), in the *Journal of Hygiene* for April, 1905.

Dr Hamilton Wright, in advancing the theory that Beri-beri is an acute infectious disease with a definite primary lesion, attaches considerable importance to the results of certain recommendations made by him in 1902 with a view to improving the health of the prisoners in the Pudoh Gaol, Kuala Lumpur.

As State Surgeon, Selangor, all practical work in connection with the treatment and care of the prisoners is carried out under my direction. I am therefore able to speak with authority as to the nature and results of any sanitary work done in the Gaol.

I regret to say that Dr Hamilton Wright is under a misapprehension as to the extent of the reforms carried out in the Pudoh Gaol since he completed his observations in 1902.

A special study of Beri-beri having been made in connection with the various outbreaks of this disease at the Pudoh Gaol since 1895 and the results of various observations having from time to time been published, it is, I think, essential, in the interest of medical science, that all statements made in connection with the progress of the disease and measures taken to control it, should be correct in every detail.

I propose therefore to quote from Dr Hamilton Wright's article, showing in each case what has actually occurred.

"*I made in* 1902 *certain recommendations with reference to the mode of life and hygienic surroundings of the prisoners in the Kuala Lumpur Gaol. More than two years have elapsed since the application of the preventive measures I advocated was begun—time enough in which to test their efficiency.*"

Dr Hamilton Wright made altogether seventeen recommendations, involving temporary abandonment of the Gaol, structural alterations in the building, demolition of the hospital infirmary, and various alterations in the habits of the prisoners. Of these seventeen recommendations one only, the enlargement of the ventilation spaces in the cells, was carried out, but as none of the altered cells were occupied until the end of January, 1903, by which time Beri-beri had practically disappeared from the Gaol, this can have had little effect in causing the decrease in the number of cases of the disease.

"*The various hygienic reforms which came into force between May and September,* 1902, *were as follows*:—

"*All prisoners were employed during the greater part of the day at extra-mural work. From September 23rd,* 1902, *they were accommodated in open-sided sheds during the day.*"

This arrangement was not suggested by Dr Hamilton Wright but was carried out by Government on the recommendation of the local medical officers, and in view of satisfactory results following similar measures undertaken in 1897 in connection with a severe outbreak of Beri-beri among the prisoners.

"*The Gaol has been thoroughly disinfected several times. In February,* 1902, *the entire building was washed with* 2 °/₀ *Formalin.*"

That there has been some mistake as to the extent of the disinfection by Formalin is shown by the following extract taken from a letter from Dr Hamilton Wright to Dr Daniels, Director of the Institute of Medical Research, Kuala Lumpur, dated June 30th, 1904.

"*The disinfection carried out in February,* 1902, *was under my direction and consisted in spraying out the Gaol with a* 2 °/₀ *Formalin solution. I went to India about the middle of the month, after seeing the disinfection well under way. Unfortunately through some misunderstanding, Formalin which lay in the goods shed was not secured and the disinfection came to an untimely end.*"

Mr Galloway, the Gaoler, who directed the disinfection work,

informs me that about one-fourth of the Gaol was disinfected with the Formalin solution.

"*Under the old régime the prisoners defaecated and urinated in their cells. The provision for the act was most primitive. A small box of sand was provided with a few thin sticks of wood for cleaning the anus. Observation soon showed me that the sticks were of small use and that the fingers were more often employed and afterwards wiped on the floor or on the bedding. It appeared to me that this particular fault in the personal hygiene of the prisoners was the chief factor in the spread of the disease.*"

The arrangements are in detail as follows :—Each prisoner has a small tin pail fitted with a tin lid in which he defaecates and urinates, he is also provided with a box of dry earth to cover up the faeces in the pail, and with the thin sticks of wood described by Dr Hamilton Wright a small quantity of a solution of Jeyes fluid is placed in each pail when it is put in the cell.

The prisoners are cleanly in their habits, and, during the thirteen years during which I have been connected with the Gaol, I have not seen any case in which the prisoners have used their fingers in the way described.

"*In consequence of my recommendation, defaecation by the prisoners in their cells has been stopped.*"

This is an unfortunate mistake. The prisoners defaecated in their cells as before, and there has been no alteration of any kind in the arrangements made for them in this connection.

"*The abolition of Beri-beri in this Gaol has, I consider, been attained by hygienic reforms founded on the view which I have advanced that the infective agent is contained in the excreta of the patient during the acute state of the disease and that infection results from faecal contamination. I would suggest that as an additional preventive measure, the stools of those suffering from acute Beri-beri be disinfected.*"

For several years the excreta of all patients, including those suffering from Beri-beri, have been passed into a disinfectant solution.

I regret that there should have been any misunderstanding in connection with this subject, more especially as I was under the impression that all had been made clear by a letter from Dr Daniels dated May 2nd, 1904, in which, at Dr Hamilton Wright's own request, complete details of any alterations that have been made in the Gaol or in the habits or the prisoners, were supplied to him.

I shall be much obliged if you will take such measures as seem to you to be suitable in order to correct the possibly misleading impression conveyed to the medical profession by the article referred to.

I have sent Dr Hamilton Wright a copy of this letter.

Believe me,

Dear Sir,

Yours faithfully,

G. A. O. TRAVERS,

State Surgeon, Selangor.

To the Editor,

The Journal of Hygiene.

PUBLICATIONS RECEIVED.

BOOKS.

Dieudonné, A. (1905.) *Immunität, Schutzimpfung und Serumtherapie. Zusammenfassende Uebersicht über die Immunitätslehre.* 4th edition. 210 pp. Leipzig: Joh. Ambr. Barth. 24 × 16 cm. Price unbound 6 marks, bound 7 marks.

The fact that this work has reached its fourth edition sufficiently indicates that it serves a useful purpose in giving a good digest of the subject of immunity, protective inoculation, and serum therapy.

Goeldi, E. A. (1905.) *Os Mosquitos no Pará.* Excerpt from Mem. do Museu Goeldi de Historia Natural e Ethnographica IV. 54 pages, 144 text figures and 5 chromolithographs. Pará (Brazil). 34 × 24 cm.

An excellent contribution, incorporating many original observations on the structure and biology of the mosquitoes of Pará. The illustrations are all original and mostly good, the coloured plates being especially excellent.

Harrington, C. (1905.) *A manual of Practical Hygiene for Students, Physicians, and Medical Officers.* 3rd edition. 793 pp. 12 plates and 118 figures. 24 × 16 cm. Philadelphia and New York: Lea Brothers & Co.

James, S. P. and Liston, W. G. (1904.) *A Monograph of the Anopheles Mosquitoes of India.* 132 pp. 10 half-tone plates, 15 coloured plates, and 12 text figures. Cloth. Calcutta: Thacker, Spink & Co. 28 × 21 cm.

A work of the first importance for those interested in mosquitoes.

Smith, F. (1905.) *A Manual of Veterinary Hygiene.* 3rd edition. 1035 pages, 225 figures. London: Baillière, Tindall and Cox. 22 × 15 cm. Price 15/- net, cloth.

This work presents a very handsome appearance and the author has taken obvious pains to bring its contents up to date even to the inclusion of Ehrlich's side chain theory.

Weyl, Th. (1904.) *Zur Geschichte der Sozialen Hygiene.* Pp. 791—1062. 2 plates and 8 text figures. 25 × 17 cm. Excerpt from Weyl's Handbuch der Hygiene. Jena: G. Fischer.

A valuable and exhaustive contribution to the history of the subject.

POPULAR.

Wakefield, H. Rowland. (1905.) *Lessons on Living. A reading book in Physiology and Hygiene.* ("Adapted for use in schools in which elementary science is taught as a class subject.") Nos. VI—VII. 240 pages. Blackie & Son (London). 18 × 12 cm. Price 1/6, cloth.

The Doctor Says. (1905.) *A book of Advice for the Household with Practical Hints for the Preservation of Health and the Prevention of Disease.* 306 pages. London: Sidney Appleton. 19 × 12 cm. Price 3/6 net. Cloth.

BROCHURES.

Caton, R. (1905.) *How to Live. A short Account, in simple words, of the Laws of Health with a brief reference to habits and conduct*, written for the older pupils in primary schools. 42 pages. London: Williams & Norgate. 18 × 12 cm. Price, paper, 3*d.*

Hoffmann, W. (1905.) *Leitfaden der Desinfektion für Desinfektoren, Verwaltungsbeamte, Tierärzte und Aerzte.* 138 pages, 88 text figures. Leipzig: Joh. Ambrosius Barth. Price, boards, 3 marks. 22 × 15 cm.

A short and practical guide to the methods of disinfection. Contains numerous new illustrations and much useful information not found in other works.

Liverpool. (1905.) *Observations of the Medical Officer of Health upon the report of Dr Reece to the Local Government Board on Smallpox and Smallpox Hospitals at Liverpool*, 1902—3. 23 pages, numerous diagrams. Liverpool: C. Tinling & Co. Ltd. 1 map. 33 × 21 cm.

Pfeiler, W. (1905.) *Zur Kenntnis der Desinfektion infizierten Düngers durch Packung.* Inaug. Dissertation. *Arb. a. d. Hyg. Institut d. Kgl. tierärztl. Hochschule zu Berlin*, No. VI. 100 pages. Berlin: Richard Schoetz. 24 × 16 cm.

Relates to the disinfection of infected manure by the process of stacking it. Records the results of previous investigations and the author's researches in the subject.

Pirquet, C. von and Schick, B. (1905.) *Die Serumkrankheit.* Leipzig and Vienna: Franz Deuticke. 144 pages, xxxiii. charts. 25 × 18 cm. Price 4.50 marks.

"Serum Disease" is a term which includes all the pathogenic effects due to the injection of foreign sera. The book is divided into three sections dealing with: I. The clinical aspects of the disease; II. The effects of repeated injection of serum; III. The theory of serum disease. A good bibliography is to be found at the end. The work contains chiefly original matter and constitutes an important contribution to our knowledge of the affection.

The Infant's Health Society. (1905.) *The present conditions of Infant Life, and their effect on the nation.* 16 pages. London: Baillière, Tindall & Cox. Price, paper, 6*d.*

Wallace, S. (iv. 1905.) *The rôle of Modern Dietetics in the Causation of Disease.* 87 pages. Cloth. 22 × 15 cm. London: Baillière, Tindall & Cox.

A collection of essays which appeared originally in the *Brit. Med. Journ.*, *Lancet*, and other Journals. The book is divided into eight chapters which refer to the causation, increase and decrease of disease; the physiology of mastication; the effects of refinement of food; nasal obstruction and mouth-breathing; physical deterioration in relation to the teeth; speculations and suggestions.

Watson, J. R. (1905.) *Natural Science in Hygiene: or the Life-History of the Non-Bacterial Parasites affecting Man.* For the use of Students of Public Health. 62 pages, 17 figs. Bristol: J. Wright & Co. 16 × 11 cm. Price 1/6 net.

REPORTS AND PERIODICALS.

Atti della Società per gli studi della Malaria. Rome. (1905.) Vol. VI., 666 pages. Numerous maps and tables.

Boletin del Instituto de Sueroterapia, Vacunación, y Bacteriologia de Alfonso XIII. edited by Don Santiago Ramon y Cajal. Madrid: A. Marzo. Vol. I., No. 1, March, 1905. 24 × 17 cm. This Journal will appear as a quarterly. The opening number contains 47 pages of printed matter: *Cajal*, Diagnostico histológico de la rabia;—*Mendoza*, Coloración de los bacillus phymogenus, de la lepra, etc. por la eosina;—*Murillo*, Vacuna anticarbuncosa T.;—*Garcia é Izcara*, Profilaxis de la viruela ovina;—*Puerta y A. Mendoza*, Analisis de las aguas potables, etc.;—*Llavador*, Tratamiento antirrábico, etc. en el Instituto de Alfonso XIII., 1904.

HILL, E. (1904.) *Report on the Plague in Natal*, 1902—3. London, etc.: Cassell and Company. 192 pages, 34 figs. Numerous charts. Cloth. 25 × 16 cm.

Hongkong. (1905.) *Report of the Acting Medical Officer of Health on the Epidemic of Plague in the Colony of Hongkong during the year* 1904. Hongkong: Noronha & Co. 69 pages, numerous charts and tables. 33 × 21 cm.

Jahresbericht über die Fortschritte und Leistungen auf dem Gebiete der Sozialen Hygiene und Demographie. Grotjahn, A., and Kriegel, F. (1904.) Vol. III. for 1903. 376 pages. 24 × 17 cm. Jena: G. Fischer.

London County Council. (7. II. 1905.) *Report of the Medical Officer, to which are appended Reports by Dr Frank Clowes (the Council's Chemist) and Dr A. C. Houston, on Watercress and Watercress beds in the Neighbourhood of London.* London: P. S. King & Son. 18 pages. Price 6*d*. 33 × 21 cm.

Memoria de los trabajos ejecutados en el Consejo Superior de Salubridad en el año de 1904. 285 pages, numerous charts. Mexico. 1905. 32 × 21 cm. (The Memorias published 1902, 1903, 1904 also received.)

NIETNER, D. (1905.) *Der Stand der Tuberkulose-Bekämpfung im Frühjahre* 1905. *Geschäftsbericht.* 311 pages, numerous plates. 29 × 23 cm. Berlin: Deutsches Central-Komite zur Errichtung von Heilstätten für Lungenkranke.

Reports (VIII. 1905) *of the Commission appointed by the Admiralty, the War Office, and the Civil Government of Malta, for the investigation of Mediterranean Fever, under the Supervision of an Advisory Committee of the Royal Society.* Part III. 98 pages, 2 plates. London: Harrison & Sons. 22 × 15 cm. Price 2/6.

Reports (1905) *of the Sleeping Sickness Commission of the Royal Society.* No. VI. 287 pages, 3 maps, 4 coloured plates, 2 text figures, numerous charts. 25 × 15 cm. Price 4/6.

Report (1905) *on Health of Animals.* Extract from Annual Report, 1904. Dep't. of Agric. Canada. Ottawa: Gov't. Printing Bureau. 186 pages, 15 figures, 18 plates. 25 × 17 cm.

Scientific Reports on the Investigations of the Imperial Cancer Research Fund. (1905.) No. 2. Part I.—*The Statistical Investigation of Cancer.* 58 pages. Price 2/6. Part II.—*The Growth of Cancer under Natural and Experimental Conditions.* 96 pages, 55 figures, 19 plates. London: Taylor and Francis. 25 × 19 cm. Price 2/6.

The Infant's Health Society. (1905.) *The Report and Statement of Receipts and Expenditure for the year ending* 31*st December*, 1905, *including the Report of the Infants' Hospital, Denning Road, Hampstead, N.W.* 19 pages. London: G. Pulman & Sons, Ltd.

Third Annual Report (1905) *of the Superintendent of the Bureau of Government Laboratories, for the period from September* 1, 1903, *to August* 31, 1904. Manila: Bur. of Public Printing. 105 pages, 1 plan, 5 charts, 8 plates, 23 × 15 cm.

Third Report (1905) *on "The Treatment of Venereal Disease and Scabies in the Army."* 57 pages. London. 33 × 24 cm. Price 1/-.

REPRINTS.

Bardi, D. R. (i. 1905.) *Adulteration of Food and Drugs in Bombay, its detection, and the best feasible means that can be suggested for its prevention.* Bombay Med. and Phys. Soc. "Transactions," vol. ix. 57 pages, 43 tables, repr.

Christophers, S. R. (1905.) *On a Parasite found in Persons suffering from Enlargement of the Spleen in India.* (Third Report.) Sc. Memoirs by Officers Med. & Sanit. Dep'ts. Govt. of India. Calcutta. N.S., No. 15. 14 pages, 1 fig., 1 plate, repr.

Freer, P. C., and Polk, M. (1905.) I. *Description of New Buildings* (by Paul C. Freer). II. *A Catalogue of the Library* (by Mary Polk, Librarian). Dep't. of the Interior, Bureau of Gov't. Laboratories. Manila. No. 22. 319 pages, 27 plates, (with Frontispiece). 23 × 15 cm.

Herzog, M. (1904.) *The Plague: Bacteriology, Morbid Anatomy, and Histopathology.* Dep't. Inter. Bur. Gov't. Labs. Manila. No. 23. 149 pages, 27 figs., repr.

Hunt, R. and Motter, M. G. (1905.) *Changes in the Pharmacopoeia of the United States of America.* (Eighth Decennial Revision.) Hygienic Lab. Bulletin, No. 23. 122 pp. Washington: Gov't. Printing Office. 23 × 15 cm.

Lamb, G. (1905.) *Snake-venoms in relation to Haemolysis.* Sc. Memoirs by Officers Med. & Sanit. Dep'ts. Govt. of India. Calcutta. N.S., No. 17. 15 pages, repr.

Lebredo, M. (vii. 1904.) *Some observations on the Anatomy of the Mosquito, with original drawings.* Revista de Medicina Tropical. Havana. 25 pages, 18 figs., repr.

McClintic, T. B. (1905.) *Chloride of Zinc as a deodorant, antiseptic, and germicide.* Hyg. Lab. Bulletin, No. 22. 24 pages. Washington: Gov't. Printing Office. 23 × 15 cm.

McGregor, R. C. (1905.) I. *Birds from the Islands of Romblon, Sibuyan, and Cresta de Gallo.* II. *Further notes on birds from Ticao, Cuyo, Culion, Calayan, Lubang, and Luzon.* Dep't. of the Interior Bur. of Gov't. Labs., No. 25. 34 pages, 11 plates. Manila: Bur. of Publ. Printing. 23 × 15 cm.

Rosenau, M. J., Parker, H. B., Francis, E., Beyer, G. E. (1905.) *Report of working party No. 2, Yellow Fever Institute. Experimental Studies in Yellow*

Fever and Malaria at Vera Cruz, Mexico. Yellow Fever Inst. Bull., No. 14. 101 pages, 2 charts, 3 plates. Washington. 23 x 15 cm.

STILES, CH. W. (1905.) *A Zoological Investigation into the Cause, Transmission, and Source, of Rocky Mountain "Spotted Fever."* Hyg. Lab. Treasury Dep't. Bull., No. 20. 119 pages, 1 chart. Washington. 23 x 15 cm.

TODD, J. B. (20. v. 1905.) *Alkaline Beverages in the Treatment of Pneumonia.* New York Med. Journ. and Philadelphia Med. Journ. 14 pages, repr.

VAUGHAN, V. C., and WHEELER, S. M. (1905.) (1) *Further studies on the intracellular bacterial toxins.* (2) *The extraction of the intracellular toxin of the colon bacillus.* (3) *The action of the intracellular poison of the colon bacillus.* Journ. Amer. Med. Assoc. 53 pages, 3 charts, 4 tables, repr.

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